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The Effects of CW-Related Chemicals on Social Behavior and Performance

Annual Report

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Summary, Abstract, or Digest

This report summarizes work accomplished in the first year of a three year project aimed at devloping a battery of tests of social behavior and performance that will be sensitive to the effects of CW-related chemicals considered for use as antidotes or prophylactics against CW agents. Procedures for assessing social behavior in nonhuman primates are described and compared. The presence and absence of correlations between social behavior and performance on two operant schedules, a test of complex problem solving, and behavior in a novel environment are reported as are the effects of caffeine (as a control) and atropine on the social and performance variables.

Foreword

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Facilities and Care and Use of Laboratory animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

Body of the Report

A. Overview:

This report describes the work conducted during the first year of a three year project which involves the development of a battery of individual tests for use in studying the effects of chemical warfare (CW) related chemicals on social behavior and performance. The specific objectives are: (1) To develop a set of behavioral tests for studying social behavior, individual performance, and the relationships between individual performance and social behavior in nonhuman primates. (2) To examine the effects of CW-related chemicals that might be used as antidotes or prophylactics for CW agents on social behavior and performance. (3) To develop procedures and provide facilities for testing the long term behavioral sequalae of non-lethal exposure of nonhuman primates to CW agents.

The first few months of the year were devoted both to training new personnel in the procedures to be used in capturing and handling the animals, collecting data on social behavior, and conducting the behavioral tests and to conditioning the adult males from the several monkey groups to the daily handling procedures. During this time, brief tests of open field behavior and responses to a novel environment were conducted in conjunction with the obtaining of blood samples from the animals for assay of plasma stress hormones; this was done both to provide training and experience for the project personnel and to give a measure of the degree to which the monkeys were adapting to the capture and handling routine. Training on three learning and performance tasks which

previously had been shown to be related to social behavior (Bunnell, 1982) was begun in the winter of 1984, using different sets of adult males from different social groups on the different tasks. This was interspersed with additional exposures to the novel environment of the open field accompanied by the collection of blood samples.

Early in the spring of 1984, six adult and subadult males were established in individual cages in the laboratory. These animals, whose primary function will be to serve as subjects for studies of cooperative behavior and laboratory tests of dydadic social interactions were tested in the open field at this time and later served as subjects in pilot experiments used to determine drug doses to be used with the other animals. Toward the end of the contract year these animals were set up as a social group and studies begun on the effects of caffeine and atropine on their social behavior.

Training of the observers in scoring social data was accelerated as the weather improved in the spring and, by the end of May, enough reliable social data had been obtained to provide a baseline for work relating laboratory performance to social variables. Through September 30, 1984, reliable data from a total of 244 hours of observations were collected in the outdoor compounds housing the monkey troops.

An experimental protocol for studies of the effects of caffeine on social behavior and peformance was submitted to the command (a copy is attached as an appendix to this report). Beginning in late June, the effects of caffeine sodium benzoate on social behavior, open field behavior, complex problem solving, and on two operant schedules were studied in a series of experiments. At the close of the contract year, this work was being extended to include the effects of atropine sulphate and atropine methyl nitrate on these same behaviors.

Work was begun on the development of an operant task that will require the monkeys cooperate with one another and a pilot project established to determine the parameters for training one set of animals on a free operant avoidance task.

An unreliable and aging laboratory computer caused problems with the operant testing and the analysis of social data. A proposal for additional funds to purchase a new computer, together with the necessary interfacing, was submitted in the early spring. This was approved in September and it is expected that the new system will be operational in the winter of 1985.

It may be noted that the primary objective of the work is the development of the battery of behavioral tests that relate social behavior to laboratory performance and which will be sensitive to the effects of CW-related chemicals. The objective is <u>not</u> the screening of CW-related drugs per se; in fact, the Request for Quotation on the contract was very explicit about this matter. In the sections which follow, reference will be made to a number of studies in which the effects of caffeine and atropine on various behavioral tasks have been examined. Some of these studies are complete experiments which yield useful information about drug effects on performance. Others are incomplete in terms of certain control and/or other

procedures that would be required of a proper, full scale experiment in behavioral pharmacology. This is because the mission of the project is primarily focused on the development of appropriate tests for inclusion in the battery and only secondarily on drug/performance relationships. In some instances, we expect to go back to the experiments and finish them up properly; other will simply be abandoned if they do not appear likely to contribute to the overall goals of the project. The work accomplished to date and a program for the work to be done during the second year of the contract will be found in the sections which follow.

B. Monkey Colony:

Animals. As of 30 September 1984, the colony consisted of 92 Macaca fascicularis monkeys (variously known as cynomolgous, crab-eating, or Java macaques). These are housed in four groups for the purpose of studying social behavior and organization. Two troops, named T-Troop and NT-Troop are breeding troops that contain all age/sex classes of animals. The third and fourth groups, I-Troop and C-Troop are both all male units. T-, NT-, and I-Troop are housed in outdoor compounds and the members of these groups are together at all times except when they are undergoing testing in the laboratory or when experimental manipulations of the social organization are being performed. These three troops have been in existence for a number of years and were the subjects of study in an earlier USAMRDC contract (see Bunnell, 1982). C-Troop was formed in the spring of 1984. It consists of young adult and subadult males that were removed from NT-Troop. C-Troop animals are housed in individual cages in the laboratory and are brought together only during social behavior testing. The composition of the various groups is given in Table 1:

Table 1

Group Composition as of 30 September 1984
(Number of monkeys in each age/sex category.)*

	Adult		Subadult		Juvenile		Infant	
	M	F	M	F	M	F	M	F
TROOP:								
"T" N=46:	6	16	3	5	1	3	7	5
"NT" N=31:	7	12	3	0	2	1	3	3
"I" N= 8:	8	0						
"C" N= 6:	4	0	2	0				

* Males (M) over 6 years old and females (F) over 4 years old are classified as adults. Males 4-6 and females 4 years old are subadults. Juveniles are over 1 year old (both sexes).

Housing. T-, NT-, and I-Troops are each housed in outdoor compounds 14.1 m long, 3.1 m wide, and 2.0 m high. Each compound is equipped with perches, swings, and a water fountain and contains an observer station, 1.6 m square, in the center from which observations of social behavior are taken. The compounds are connected to heated and airconditioned indoor quarters by runways that are 1.2 m in cross section. The runways are partially covered to provide shelter from rain and sun when the animals are outside. The indoor quarters are cages 6.1 m long x 1.2 m wide x 2.0 m high which are equipped with water fountains and perches. Small guillotine doors on the sides of these cages are used to collect the animals in transport boxes for testing in the laboratory. Guillotine doors between the indoor cages and the runways, and between the runways and the compounds, allow the animals to be moved to different sections of the living quarters during social testing and daily cleaning.

The 6 males of C-Troop are housed in a battery of individual cages in a separate colony room in the laboratory. An adjacent suite contains a cage, measuring 1.8 m x 1.8 m x 1.8 m, in one room and an observer station, equipped with one way windows, in the other. The C-Troop monkeys are brought from their colony cages and placed in this cage for studies of activity and social behavior. This cage will also be used for the studies of cooperative behavior that are being developed.

Yet another room contains a battery of 18 individual cages that are used as a holding facility during laboratory testing.

Adaptation to capture and handling. The behavioral testing performed in the laboratory requires that the monkeys serving as subjects be removed from their social groups each day, weighed, and brought to the test apparatus. They also must be adapted to the restraining device used to hold the animals while blood is drawn for assay for stress hormones. This adaptation process was begun in mid-November, 1983 for the I-Troop animals and was extended to the T- and NT-Troop adult males in January, 1984 and to the animals in the newly established C-Troop in March. The procedure is now a part of the daily routine for all animals undergoing experimental testing. A series of blood draws for assays of plasma cortisol and plasma prolactin have been taken throughout the year, beginning in December, 1983. These will be used to monitor adaptation to the capture and handling process and to study the affects of social and laboratory induced stressors. (Assay data from these samples should be available for inclusion in the final draft of this report.)

Health; deaths and births. TB skin testing of the entire colony was conducted in fall, 1983, and winter, spring and summer, 1984. All animals were negative. A mild outbreak of shigella occurred during the wet, cold winter months, but all animals were clear of symptoms by spring. The recurrence of diarrhea in several animals in August prompted us to have cultures for shigella and salmonella done on the entire colony.

Twenty six shigella carriers, primarily females and juveniles, were identified, treated, and recultured. Although this has solved the immediate problem, we will swab the animals again this winter and again every six months to monitor the situation. The floor in the indoor colony cages has begun to peel, and the University of Georgia has made funds available to remove the old flooring material and reseal the concrete. This will improve sanitation and should help keep problems to a minimum. Between 1 October 1983 and 30 September 1984 there were five deaths in the colony; a newborn infant died of unknown causes, an older infant of head injuries received when its mother was being captured for TB testing, and a juvenile male was found outside in February suffering from hypothermia; autopsy revealed gastric ulcers and hepatic hemosiderosis. In January an adult male from T-Troop died; the necropsy report showed diffuse acute peritonitis and necrohemorrhagic cystitis. In February, a maintenance man allowed an adult male from NT-Troop to escape; this animal did not return to the compound that day and subsequently died of exposure to subfeezing temperatures during the following night. There were a total of 14 live births in the colony in 1983 and 12 of these have survived. Between January and the end of September, 1984, there were five more births and all these infants have survived.

C. Activity Tests and Drug Dose Selection:

Procedures for observing general activity and for selecting the initial doses of the drugs to be used in the project were developed and standardized using the C-Troop males. The animals are released individually into the C-Troop observation cage (described earlier) and observed through the one-way glass windows. Locomotor movement within the cage, which is divided into 8 imaginary 2 m cubes, is recorded by the observer who also records the behavior of the animal using a rating scale similar to that used in scoring social behavior that is described in the next section - the animals often interact with their images in the one-way glass. The rating scale also contains additional codes for various behaviors that are directed toward the environment. After 10 min, the observer dons a rubber fright mask and enters the observation room. Activity and behavior in response to the masked observer are recorded for 90 sec. The test is concluded by having the observer wave a length of garden hose in front of the monkey. In establishing the initial doses of drugs to be employed in the social and performance tests, the monkeys are observed for an hour or more, beginning immediately following injection of the drug. In these observations, several fright mask presentations are made at intervals throughout the period. In addition to recording activity and behavior, the observer notes all physical changes as they appear, such as changes in respiration pupillary dilation, speed and coordination of movements, etc. The monkeys are then returned to their home cages and monitored by an observer until all overt signs of drug effects have returned to normal. The animals are given food and water at this time and the latencies to eat and drink

are recorded, as well as the kind of food that is eaten first (monkey biscuit, vegetable, fruit, etc.). In these tests, the onset of overt behavioral and physiological changes is used to determine the time that will be used between administering a drug and the beginning of any behavioral test.

As soon as the observation cage was completed in early June, a series of activity tests were run with C-Troop males that had been given various doses of caffeine sodium benzoate. Increases in locomotor exploratory behavior were noted at the 3-4 mg/kg range; depending on the animal involved, both increases and decreases appeared in the 10-16 mg/kg range, and there were no overt changes in the 0.6-1.0 mg/kg range. On the basis of these observations, our dose selection for the initial experiments was 0.8, 4, and 12 mg/kg of the salt. The smallest dose was used because it is supposed to be in the benzodiazepine antagonist range and we are to work with diazepam later in the project - see the appendix (p.5) for details. Later in the summer, because of the individual differences we observed in the effects of caffeine on performance and because we were a bit concerned about tolerance, additional doses, including 2, 8, 16, 24 and 36 mg/kg, have been added to some of our protocols. Effects appeared with a short latency - on the order of 5-10 min - and 5 min was selected as the latency to be used between injection and the behavioral tests. In the pilot work with atropine sulphate, doses of 0.8, .20, and .40 mg/kg produced maximum pupillary dilation and changes in respiration rate at about 15 min. post injection. The two highest doses produced dose dependent decreases in activity; however, the animals movements were well coordinated and they responded normally to the presence of the masked figure, giving lip smacks, some threats, and a lot of flight and avoidance behavior.

When the monkeys were returned to their home cages and fed, they showed an immediate interest in food and would eat fruit immediately, followed by pieces of sweet potato. Dry monkey biscuits were nibbled, but not consumed until two or more hours after return to the cage. Interestingly, though they ate moist food, they did not drink water immediately, nor was there any prolonged drinking at any time. It is as though they preferred the moist food because their mouths were dry, but there was no evidence of a centrally motivated thirst at these doses. Pupillary dilation typically lasted for several hours after return to the home cage, and this was the most persistent physical sign we observed. The first studies of the effects of atropine sulphate on performance utilized doses of 0.8, .20, and .40 mg/kg with a delay of 15 min between injection and testing. Subsequently, we have added a dose of .032 mg/kg and dropped the .40 mg/kg dose. We are also using delays of 30 and 60 min in some protocols.

D. Social Behavior and Organization:

During the year, considerable social data were collected from all of the monkey groups using both focal animal and group scan techniques during the same observation periods. These data are being analyzed and compared directly in order to help us select the best procedures, or combination of procedures, for identifying drug effects while at the same time maximizing our chances for detecting correlations between social behavior and learning and performance variables. We also began to look at dyadic social interactions between the C-Troop animals that were placed together only during social testing.

Group social behavior. Observations of social behavior are done using the behavior categories given in Table 2. The observers records the code for the animal exhibiting the behavior, a code for the behavior itself, and then a code for the animal that is the recipient of the behavior. Data may be recorded either by pencil and paper and later punched into a tape that can be read by a laboratory computer, or entered directly into a portable single-board computer, stored on cassette tape, and then punched onto paper tape for analysis.

Observations utilize both "group scan" and "focal animal" techniques. In a group scan, the observer watches the entire group and records every behavior that occurs as it happens; a modified version of a group scan involves looking at each monkey in sequence and recording what it is doing at the instant it is scanned. The focal animal procedure involves attending to only one animal for a period of time and recording the direction and nature of all behavior it either does or receives during that time. During the past year, we have used a combination of scan and focal techniques in most of our observations. With this procedure, each observation period begins with a 5 or 10 min scan, depending on the troop being observed. This is followed by a series of 5 min focal observations during which each adult male is the subject of a focal period - the order in which each focal animal is observed is changed each day. The observation period is concluded by another group scan.

The behaviors which each animal directs toward every other member of the troop and the behaviors which it receives from every other member in its troop are used to construct matrices which summarize the dyadic interactions in each group. These are then used to define and analyze the social organization. A very important element of the social organization of the primate groups is the presence of dominance hierarchies. The adult males have such a hierarchy among themselves and each animal's social rank within this hierarchy is determined by defeats. The occurrence of a submissive behavior in a monkey indicates that the monkey is inferior in rank to the animal toward which the submissive signal is directed. Knowledge of each male's status with regard to all of the other males defines the hierarchy.

Table 2

M. fascicularis Behavior Categories

Agonistic Behaviors:

Aggressive
- Chase
- Threat (open-mouth)
- Charge
- Slap
- Bite

Submissive
- Avoid
- Grimace
- Squeal
- Flee

Other Agonistic
- Lid
- Lip Smack
- Enlist
- Demonstrate

Sexual Behaviors:
- Sexual Present
- Mount (no thrusting)
- Mount (with thrusting)
- Masturbate
- Genital Manipulation (other animal)
- Genital Sniff (other animal)

Other Social Behaviors:
- Present to Groom
- Groom
- Ventral-Ventral Hug
- Ventral-Dorsal Hug
- Sit-Next-To (Physical contact)
- Play (not included in analysis)

Non-Social Behaviors:
- Self Groom
- Move
- Sit - No Social Interaction

Another critical element in this species' social organizaton is the hierarchy of matriarchies, such that each female and her daughters are a social unit and each such unit has a social rank within the troop. The means by which one animal establishes and maintains dominance over another (e.g., by attack, threat, teaming up with another animal) varies from animal to animal, from group to group, and from situation to situation. By recording and analyzing the entire range of social behavior in our animals we define both the behavioral constancies and the range of variation of each of our subjects. This provides a more detailed picture of social status and social organization than a simple assignment of rank.

In analyzing social behavior, the group scan data are summarized by a laboratory computer which provides a listing of the frequencies of each behavior performed by each monkey and the frequencies with which it directs these behaviors to each of the other monkeys in the troop. These data are then used to produce a series of matrices which describe the basic social organization and dynamics of the group. Usually, several days' data are combined in these analyses. In this procedure, the computer goes through all of the data and determines the social rank of each animal on the basis of who is defeated by whom, using the submissive behavior categories listed in Table 2. It then prints a series of six matrices , using the same social rank order, or dominance hierarchy, it determined from the analysis of the submission scores. In each matrix, the frequency of occurrence of each behavior, or class of behaviors selected for inclusion in that matrix, is given for each animal with respect to every other animal in its troop. (Presently, we are limited to 24 x 24 matrices; in scoring the behavior in T- and NT-Troops this year, the behavior of the 23 oldest animals in each group was scored and the 24th slot was used to represent all the remaining infants and juveniles in the troop). Four of the six matrices are used to summarize the combinations of behaviors listed under the functional categories Aggressive, Submissive, Sexual, and Other Social as given in Table 2. For the other two matrices, any individual behavior of interest may be selected. Thus, we might look at contact aggression in order to compare it with the matrix for overall aggression, or obtain separate matrices for grooming, which is included in the Other Social matrix and play, which is not. Examples of these matrices may be found in the proposal for this project and in Bunnell (1982).

The data from each focal animal observation are analyzed individually, using the computer program employed with the initial analyses of the group scan data. These then can be summarized across observations to provide baseline information to which the data from observations during experimental manipulations can be compared. Use of the focal animal observation procedure is essential for the study of drug effects on social behavior since it ensures that each subject is observed in the same way, and for the same length of time, during each session. The procedure does has disadvantages.

however, in that social interaction between other members of the troop are not recorded. Information about such interactions is often critical for achieving some of the other objectives of the contract, so we have utilized both scan and focal procedures in all of our observations during the past year.

In our previous work, we have made only limited use of the focal animal procedure in a study of affiliative social behavior in these groups (Perkins, 1982). A major objective of the initial stages of the work is to compare and contrast data obtained by the scan and focal animal techniques to determine the best combinations of the procedures for use in each aspect of the project; e.g. detecting the effects of a drug on social behavior may require a different strategy in setting up the observation procedures than when the objective is to relate an experimental manipulation of the social dominance hierarchy to performance on an operant task. Between late May, 1984, when observer training was essentially complete, and the end of September, a total of 244 hours of observation of social behavior were recorded for T-, NT-, and I-Troops; each period contained observations obtained from both scan and focal procedures as described above. These data, together with additional social data to be gathered this fall before cold weather arrives, will be analyzed in detail during the coming months with the objective of deciding the optimum procedures for each aspect of the project. In addition to extensive baseline data on social behavior in each of the three troops, the data include the initial studies of the effects of caffeine and atropine on social behavior as well as observations of behavior during both experimental manipulations of the social structure of the groups and while spontaneous changes in social rank have been occurring. Specific questions being asked of the data involve:

1. The extent to which the social behavior matrices described earlier are equivalent when they are generated from data using focal animal as opposed to group scan techniques. Included in this question are subsidiary questions such as the number of focal observation periods in which only the adult males are observed that are required to define (a) the male dominance hierarchy in the troop and (b) the social ranks of the other animals in the troop that interact with the focal males. A related question is the extent to which a change in the frequency of specific behaviors throughout the troop is accurately reflected by the frequencies of this behavior obtained from the focal data; yet another is the identification of those behaviors that may not be picked up at all using the focal procedure.

2. The relative sensitivity of both procedures for detecting short term changes in the social structure that may be induced by either removing or replacing animals in the troops or by administering a drug.

3. The frequency with which observations of either kind must be made in order to maintain an accurate picture of the social organization of the troop and

provide a baseline against which the experimental manipulations can be imposed. Gathering these data is a very labor intensive operation and we are interested in determining the most efficient schedules for each experimental objective of the project.

The answers to these questions will be used to set up the social observation schedules and procedures to be used in obtaining data during the second year of the project. Analyses of the data will be completed prior to the return of warm weather and the resumption of regular social testing in the outdoor compounds. (Social testing in the outdoor compounds continues during the winter months whenever possible, but cold temperatures and bad weather make the systematic collection of data very difficult during January, February, and much of March. Our strategy during this time will be to obtain enough data to monitor the social organization of the troops while utilizing the remaining time for data analyses and for working with the animals in the laboratory.)

Changes in the social rank and behavior of the males and in the social organization of the troops may occur spontaneously or they may be induced by manipulating the membership of the group under study. Experimental manipulations may be accomplished by removal of one or more animals, either temporarily or permanently, and by introducing new animals into a troop either from outside the colony or by transferring monkeys between troops. For example, removal of a high ranking male typicaly produces a reshuffling of the dominance order among the remaining males; returning the male to the original troop then results in another readjustment of the social structure. Such procedures intensify agonistic interactions between animals and are used in studying relationships between performance and aaggressive and submissive behaviors and to induce social stress within the groups.

During the year, the procedures utilized involved the removal and replacement of males within a troop. During August, the second and fifth ranked males in T-Troop were removed for 8 days as was the third ranked male in NT-Troop and the alpha (first ranked) male in I-Troop. Only the last manipulation produced a significant change in social organization as the third ranked male moved to the alpha position. The former leader reassumed first rank upon his return with very little overt aggression occurring. In September there was a spontaneous change in the dominance hierarchy in T-Troop. The third ranking animal defeated the alpha male and moved to first; the former alpha dropped to second, the former second to third, and the remainder of the animals stayed as before. On September 24 the alpha male in NT-Troop was removed. This produced a sharp increase in agonistic behavior within the group and a disruption of the social structure. The dominance structure had not restabilized by the end of the month. The former alpha male will be reintroduced after an absence of three weeks. Relationships between social behavior during these

changes in composition and structure of the groups and performance on various laboratory measures of learning and performance will be described in the sections dealing with each of the laboratory tasks.

Social behavior in C-Troop. C-Troop consists of 4 young adult males and 2 subadult males. Five of these animals are being used to study social behavior in the large cage described earlier under the section on activity and drugs. The sixth is not a part of the group per se, but is to be used as a social stimulus for the other members of the troop. The C-Troop males originated in NT-Troop. Five males were removed from NT-Troop in mid-May and housed in individual cages in a colony room assigned for that purpose; the social stimulus animal was taken out of NT-Troop in mid-August and housed individually in the same room as the other monkeys. Adaptation to the social test cage was begun in June during the activity study used to establish the caffeine sodium benzoate doses to be used with in the drug studies on the males from the other troops. Two additional weeks of adaptation, coupled with pilot studies of atropine sulphate doses on activity, were given in July. Tests of social behavior were started at the end of July and continued through the end of the contract year by which time 33 days of social data had been collected. Tests of caffeine and atropine effects on social behavior in C-Troop began during the last week of September are scheduled to continue throughout the month of October.

The initial results of the tests of social behavior in the C-Troop monkeys have been disappointing. One of the purposes in establishing the troop was to provide subjects for tests of social interactions between pairs of animals. The idea was to pair each monkey with each of the other four monkeys for 10 min a day, making a total of 10 observations. The 10 sets of relationships between the five individuals could then be studied, one at a time, under laboratory conditions and related to performance on the cooperative task being developed. Drug effects on the different sets of relationships could be examined, and a social dominance hierarchy derived from the dominance-submission relationships between members of each pair. The effects of experimentally manipulating the dominance relationships between one pair of animals on the other pairs could then be examined. Results from these tests of dyadic social interactions in the laboratory that are of particular interest could then be tested in the more natural social environment provided in the two breeding troops. The availability of a laboratory procedure would mean that the study of social behavior would no longer be totally dependent on fair weather testing in the outdoor compounds.

Observations of pairs of animals in the indoor social cage were done at the end of July and during the first week of August. Despite the fact that the animals had been away from all physical contact with each other or any other monkeys for over two months, there was very little interaction between the animals and virtually no agonistic behavior took place. The decision was made to put all five monkeys together once a day

and observe them as a group. Then, once the relationships between pairs within the group were firmly established, testing in pairs could be resumed. Accordingly, the animals were observed using the same procedures employed with the outdoor studies - a 5 min group scan was followed by a set of 5 min focal observations on each animal and the session was then concluded with another 5 min scan. The monkeys were observed throughout the rest of August using this procedure. Attempts were made to increase interactions by throwing food into the cage and by placing a feeder in the cage and observing the animals while hungry. Using these procedures, we have been able to determine the dominance relationships between the animals, but there has been very little overt aggression in the group. The animals were kept apart and not tested for one week at the beginning of September; they were retested three times during the next week, and then not tested for two more weeks before beginning the caffeine/atropine study on September 24. The purpose was to see if these short periods of social isolation would enhance the amount of interaction in the group. They had very little effect.

Despite the limited amount of agonistic behavior in C-Troop, experiments are underway to investigate the effects of caffeine and atropine on the behavior of these monkeys in the group situation. This is being done because we feel that the drugs might produce an increase in interanimal interactions and that the possibility of detecting such charges against the background of minimal social interaction is worth investigation. These experiments will give us an opportunity to evaluate this social observation procedure as an instrument for studying drug effects. The first experiment is underway utilizing caffeine sodium benzcate doses of 4 and 12 mg/kg, and atropine sulphate doses of .20 and .08 mg/kg. Physiological saline is used as the control. The animals are tested 5 days per week. On Mondays, all monkeys receive saline; on subsequent days two animals receive a drug while the alpha male and the other two get saline. Thus, on a Tuesday, the second and third ranked animals get the drug while the alpha and the fourth and fifth ranked get saline while on Wednesday, the fourth and fifth ranked monkeys get the drug while the top three animals in the hierarchy get saline and so on throughout the schedule. After this initial study, we will decide whether or not to use the drugs with the alpha male. Additional doses will be utilized if necessary, and the delay between injection and testing will be varied using delays of 5 min, 30 min, and 60 min. An experiment to compare the effects of atropine sulphate and atropine methyl nitrate in these animals has been scheduled for later in the fall using doses of .032, .08, and .20 mg/kg doses of both drugs.

During the winter we will return to our efforts to develop a better laboratory test of social behavior. In one procedure, the sixth monkey assigned to the troop will be introduced midway through the observation session and the responses of the group to the intruder will be evaluated. In conjunction with this, blood samples will be obtained during periods of no social testing, following a regular social test, and after the

session in which the intruder is introduced. Plasma prolactin and cortisol will be assayed in this pilot study to provide an index of stress under the three conditions. Following the extended testing of the group in the fall, we will reevaluate the procedure of testing the animals in pairs to see if it now apears worthwhile. A third approach to laboratory testing of social behavior in these animals will be to study triadic relationships. De Waal, van Hooff, and Netto (1976) have called attention to the importance of the participation of more than two animals in a single agonistic social interaction.

Enlisting behavior, in which one monkey solicits the aid of another against yet a third animal using characteristic gestures and postures, is an example. A positive response on the part of the solicited animal provides an instance of true cooperative behavior between the monkeys. As the development of tests of cooperative behavior is one of the objective specified by the contract, we will attempt to determine the factors which induce enlisting behavior using the C-Troop monkeys. If we can bring the behavior under experimental control we will have a powerful tool for further research.

Caffeine effects on social behavior. During July, a study of the effects of caffeine sodium benzoate on social behavior was conducted with the adult males in T- and I-Troops. Doses of 0.8, 4, 8, and 12 mg/kg were alternated with days on which the animals received injections of physiological saline. On each day, half of the males in T-Troop received caffeine and the other half received saline. In I-Troop, the alpha male and the bottom ranking male received saline injections on all days; 3 of the remaining 6 males alternated with the other 3 males in receiving drug or placebo each day. The males were removed from their troops, weighed, injected, and returned to the compounds for observation of their social behavior using the combination of group scan and focal animal procedures described earlier in this section. The order in which the doses were administered was 4, 8, 0.8, and 12 mg/kg. Each male tested with drug received one administration of each dose.

Caffeine had no discernible effect on social behavior at any of the doses employed. Data from the group scan and focal animal procedures were analyzed separately and in combination but did not reveal any consistent changes in behavior. A fight took place between the second and third ranked animals in T-Troop on the day they both received the 4 mg/kg dose, but it is highly unlikely that this was a drug effect. No changes in frequencies of agonistic social, "other" social, or nonsocial behaviors were seen in these, or any of the other animals, at any dose of caffeine. The results from I-Troop, in which the top and bottom ranked animals were not given caffeine, were essentially identical. There was no overall increase or decrease in social behavior and agonistic behavior, which was at a very low level on saline days, did not increase at any dose of caffeine sodium benzoate.

A study of the effects of atropine sulphate on social behavior in the I-Troop males is scheduled for the fall, using

doses of .032, 0.8, and .20 mg/kg in a design similar to that used in the caffeine study. When this is completed, we will decide if it will be worthwhile to do another social study with caffeine; we would use one or two higher doses - up to 36 mg/kg - of the salt and combine the drug with an experimental manipulation of the social group to produce an increase in agonistic behavior against which to study the drug effects.

E. Open Field Testing:

Open field testing is conducted to study the monkeys' willingness to enter a strange environment, the amount of exploration that they do in that environment, and their responses to stimuli, either inanimate objects or other animals, placed in the field during testing. Earlier work with this test situation (see Bunnell, 1982) showed a relationship between scores in the open field and social behavior during initial, but not subsequent, behavior in the situation. In accordance with the specifications of the contract, the test received further evaluation during the present year.

Testing is conducted in a square open field, 3.7 m on a side and 1.8 m high that is located in a large room in the laboratory building. Walls and floor are painted white, and the floor is divided into 16 squares by a painted grid. Five threaded studs, one in the center and the other four arranged in a square pattern equidistant from the center and the walls, are imbedded in the floor. These are used to attach the novel objects that are used as stimuli in some of the tests. The open field is covered by chain link fencing and is illuminated by four 150 watt floodlights placed above the ceiling. There are two guillotine doors located at diagonally opposite corners of the arena by which animals may be introduced into the field. An elevated platform located along one wall outside the arena is used for observing and scoring behavior. Opaque curtains and a one way window prevent the monkeys from seeing the observers during testing.

Monkeys being tested are brought to the open field in transport cages; these cages are placed outside a guillotine door to the arena for 5 min before the door is opened and the animal allowed access to the field. In a typical test, the animal is allowed 15 min to emerge into the field. (On some tests, if this time is exceeded, the animal is gently forced into the field and the test is continued). "Emergence" requires that the animal enter the arena and move beyond the first square in the field (a distance of @ 1 m). When the animal has emerged, the guillotine door is closed behind it and its behavior during the ensuing 5 min is recorded by the observers. At the end of 5 min, the guillotine door is reopened and the monkey is allowed to return to its transport cage. When the animals are tested in the bare field, without novel objects being present, the following measures are taken:

(1) Head Out Latency: Time from opening the guillotine door until the animal pokes its head through the door into the arena.

(2) Body Out Latency: Time from opening the guillootine door until the animal enters the square of the arena that is directly in front of the guillotine door.

(3) Number of Returns: Number of times monkey returns to transport cage after entering the first square ("body out").

(4) Emergence Latency: Time from opening the guillotine door until the animal "emerges" as defined above.

(5) Exploratory Moves: Number of squares traversed by the animal during the 5 min following its emergence into the field.*

(6) Return Latency: Time from reopening of the door following the 5 min exploratory period until the animal reenters its transport cage.

(7) Return Moves: Number of squares traversed during the return latency period.*

* Time spent on the floor is differentiated from that spent moving about on the ceiling during these periods.)

When novel objects are present in the arena, the frequencies of occurrence of the following additional behaviors are also recorded:

(8) Lip Smacking
(9) Orientation toward object(s)
(10) Manipulation of object(s)
(11) Threats toward object(s)
(12) Bites (object)
(13) Other contacts with object(s)
(14) Vocalizations
(15) Self directed behaviors (groom, masturbate, etc.)

If two or more animals are observed simultaneously in the open field, the social behaviors listed in Table 2 are also scored for both animals.

The eight adult males in I-Troop were tested during December, 1984 using both the bare open field and the field with novel stimuli in place. Although the the tests were conducted primarily to train observers in the testing procedures, the nine days of observations produced useful baseline information on these animals. During the winter of 1984, the I-Troop males were retested in order to examine the stability of their responses across time. They were given 3 days exposure to the bare field followed by 2 days with novel objects present. Six males from T-Troop and 8 from NT-Troop were also tested at this time under the same schedule of 3 days of empty field followed by 2 days with a novel object in the field. During both the December and February tests on I-Troop and the February tests with NT-Troop, blood samples for plasma hormone assays were collected in conjunction with the open field tests.

The tests with I-Troop showed that the amount of locomotor activity was fairly stable within individuals across the two tests which were separated by 66 days. Introducing the novel

objects depressed activity on the first day in December, but had no obvious effect in March (the same objects were used). There was a high positive correlation (rho = +.86) between amount of activity and social rank in the male dominance hierarchy. At the time the tests were made, however, the observers were still learning to score social behavior and enough reliable data to allow us to do a detailed analysis of this relationship is not available. A similar relationship between rank and activity was seen in the 4 T-Troop males that voluntarily emerged on each day of testing. Unfortunately, the second and third ranked animals did not emerge within the 15 min criterion period on most days. In NT-Troop, however, there was no relationship between rank and activity. The second ranked animal did not emerge on any day, however, and the third ranked animal was ill and could not participate in the tests. When these animals were retested in the summer (see the caffeine experiment below), the two top ranked monkeys were the least active and the third ranked was the most active. Clearly, the relationship found in I-Troop does not hold for NT-Troop.

In March and again in May, 1984, the five original members of C-Troop were given a single exposure to the open field with a novel object present. Three baseline blood samples and one postexposure sample were collected in each replication. The tests were run before the animals were reunited as a social group, so no comparisons of social data with open field data could be made. On the second test, the amount of activity dropped sharply in three monkeys, stayed the same in one, and increased in one. Interactions with the novel object declined from a mean of 8.2 to 1.2 across the tests (The same "novel" object was used in both tests.) Plasma beta-endorphin levels increased in 3 of 5 monkeys after exposure to the open field on the first test. Plasma from the second test is currently being assayed for prolactin and cortisol. (Results should be available for the final draft of this report.)

Effects of caffeine on open field behavior. In July and August, an experiment was conducted on the effects of caffeine on behavior in the open field using the seven NT-Troop males. In this study, the animal was given 5 min to enter the field after the door was opened. If it failed to do so, it was gently pushed into the arena and the test continued with the usual procedures. Caffeine sodium benzoate or control injections (physiological saline) were given immediately before beginning the 5 min holding period prior to releasing the monkeys into the field. Doses of 0.8, 4, 12, and 16 mg/kg were used in tests in both the bare open field and with novel objects present. One additional test with a dose of 24 mg/kg was done in the bare field. All monkeys were tested under all conditions except for one who was ill on the day it was to receive the 16mg/kg dose in the empty open field. In the tests of responses to novel stimuli in the open field, eight different objects were used as the novel stimuli. The objects were arbitrarily divided into sets of two. On a test day, four monkeys would be exposed to one object in a set and the other three to the other object of that set. The next day, each

monkey was exposed to the object in the set that he had not encountered before. This was continued until each monkey had been exposed to all eight objects over eight days of testing and had received 4 caffeine doses and 4 saline injections. The order in which the drug doses were given was 4, 12, .08, 16, and 24 mg/kg in the tests in the empty field and 16, 4, 12, and .08 mg/kg with the novel objects. Tests in the bare field with the 16 and 24 mg/kg doses were done after the tests with novel objects had been completed.

The activity scores of the animals are summarized in Table 3 which also lists the social rank of each male. Data from the tests in the bare field are given in 3a and from the novel object tests in 3b.

Table 3
Locomotor Activity Under Caffeine Sodium Benzoate

a. Empty Open Field:

Animal	Rank	Saline Mean 6 Tests (+/- SEM)	Caffeine (mg/kg) 0.8	4	12	16	24
BARKER	1	19.8 (3.1)	16	23	32	46	25
EJU **	2	7.7 (3.9)	10	5	20	6	2
WEED	3	123.8(17.0)	96	168	170	111	64
ALLEN	4	45.3 (6.6)	10	70	75	34	54
TAG	5	57.5 (9.8)	68	46	58	54	53
HOBBIT	T 6.5	66.8 (3.0)	58	102	88	70	91
KUKLA	T 6.5	33.0 (3.3)	30	18	20	44	75

b. Novel Object Present:

		Mean 4 Tests					
BARKER	1	19.8 (5.1)	21	17	12	23	-
EJU **	2	12.8 (3.6)	7	14	8	16	-
WEED	3	96.5 (6.2)	67	128	71	109	-
ALLEN	4	30.3 (2.4)	32	34	47	34	-
TAG	5	44.8 (9.9)	24	53	28	58	-
HOBBIT	T 6.5	71.5 (4.0)	61	66	75	64	-
KUKLA	T 6.5	35.0 (4.8)	24	26	33	24	-

In the bare open field condition, locomotor activity increased significantly at one or more doses in 6 of the 7 monkeys. There were considerable individual differences in the dose response curves between animals. The exception, Tag, was one of two animals that showed considerable variation in his activity between the 4 saline days prior to the tests with the novel object and the 2 saline days after the tests with the novel object. His mean activity score for the first 4 saline days was 46.8 (+/- 1.7) compared with 57.5 (+/- 9.8); thus, his activity was increased at the 0.8 mg/kg dose and the scores at the two highest doses were actually below the mean of the last

2 placebo days, which was 79.0. In all but two cases, the greatest increases in activity occurred with intermediate doses of caffeine, suggesting the presence of the U-shaped curve which the literature had led us to expect. Placing a novel object in the open field had a small, inconsistent effect on locomotor activity under placebo conditions (saline column of part a compared to part b of Table 3). The effects of caffeine were highly variable and there is no consistent pattern discernible. Perhaps there are competing response tendencies between locomotor activity and visual attention to the novel stimuli which in turn are interacting with individual differences in responsiveness to the drug.

Analysis of the individual dose response curves for emergence latencies in the bare field showed these scores to be substantially shorter at one or more doses of caffeine in 4 of the 6 animals. (Eju did not emerge voluntarily on any caffeine day and did so on only one of the six saline days.) The other 2 monkeys' latency scores were not affected by caffeine except that one exhibited a much longer latency at the 24 mg/kg dose. As compared to the first 4 days of saline injections, mean emergence latencies of all 6 monkeys were shorter and variability was much reduced on the two placebo days following the tests with novel objects. Thus, the animals entered the bare open field more quickly after being given a number of experiences. With a novel object present in the field, the emergence latencies on the saline days (mean = 3.5 sec +/- 0.3) did not differ from the latencies on the last two tests in the bare field (mean = 4.2 sec +/- 0.6) in the 6 monkeys that always emerged voluntarily. There were no consistent changes in emergence latencies in the tests with a novel object. Three of the 6 showed substantial increases in latency following one or more doses of caffeine, but the others were unaffected or had slightly shorter emergence times at one or more doses.

In tests with the novel objects, scores were obtained on the total number of interactions with the object, the number of noncontact (orienting) responses, nonaggressive contact responses (sniffing, manipulating, sitting next to), total aggressive responses (biting, threatening, etc.), and total contacts (a combination of aggressive and nonaggressive contact scores.) No fear or submissive responses were seen during these tests. Total responses to the novel object increased at one or more doses of caffeine in 6 of the 7 animals; 5 of these made the most responses to either the 12 or the 16 mg/kg dose, while the 6th peaked at 4 mg/kg. The frequency of orienting responses tended to be unchanged by caffeine - the increases were in contact and aggressive responses.

Although there was no relationship between absolute scores on any of these measures and the social status of the animals, there was a very high positive correlation (rho =+.89) between rank in the male dominance hierarchy and the percentage of interactions in which the animal made some sort of contact with the novel object. Thus, a greater proportion of responses to the novel object by high ranking animals involved physical contact with the object that was the case for lower ranking animals.

Status of open field testing. It is disappointing that the relationship between social rank and activity that was found in I-Troop and which also may be present in T-Troop, is absent in the NT-Troop males, because the tests in the open field situation are clearly a sensitive indicator of drug effects. An experiment on the effects of atropine sulphate and atropine methyl nitrate on open field behavior is scheduled for the fall using NT-Troop males in the empty open field. Once this is completed, using doses of .032, .08 and .20 mg/kg of both drugs, a followup will be done using novel objects (a different one each day) to try to confirm the relationship between object contacts and social status that was seen in the caffeine study. The two studies will be of considerable interest since an experimental manipulation of the social status of the animals has taken place since the caffeine study was done. The next round of experiments using the open field will begin after the atropine studies are completed. This will involve the use of social stimuli (strange vs familiar monkeys on different days). Baseline data will be obtained from the males in both I- and NT-Troops to be sure that the results are generalizable across groups before beginning experiments on drug effects.

F. Complex Problem Solving:

The six oldest adult males in T-Troop were trained and tested on an object quality - reversal learning set task. Performance on this task was related to social behavior and status and the effects of caffeine sodium benzoate and atropine sulphate on performance were investigated. Three of the animals had previous experience on the task and three were experimentally naive. Because of the potential importance of this task for the test battery, particular attention was paid to the course of training on the problems, and to the ease with which the animals could be retrained following breaks in testing such as those that will be encountered when these monkeys are used on other tasks in the test battery.

On this task, which is conducted in a modified Wisconsin General Test Apparatus (WGTA), the monkeys are trained on a series of 10-trial object quality learning set problems until they reach a criterion of 17 correct trial two responses in 20 consecutive problems. Reversal training is then initiated. In this condition, the animals are given four new problems each day, with lengths of 10, 11, 12 and 13 trials. (The order of presentation of problems of different length is counterbalanced across days). Reversals occur on the fifth trial of the 10-trial problems, the sixth trial of the 11-trial problems, etc. When a reversal takes place, the object that has been correct up to that trial of the problem is no longer rewarded and the other object of the pair now becomes the correct stimulus for the remaining five trials on that problem. Criterion performance is 17 out of 20 correct critical trial responses in 20 consecutive problems. The critical trial on a problem is the first trial after the reversal trial. The intertrial interval is 30 sec and the monkey is allowed a

maximum of 10 sec to respond to each stimulus presentation. There are a total of 46 trials per daily session and each session is 25-30 min long.

Measures of learning and performance obtained on this task are: Habit Formation - the intraproblem performance on each new problem up until the reversal trial is given, measured as the number of correct responses on initial learning of each day's four problems. Concept Formation - assessed on both the object quality learning set and the reversal learning set portions of the problems. Correct responses on the second trial of each new problem across successive problems constitute the measure of object quality learning set performance and correct responses on the critical trials (above) across problems are the measure of reversal learning set. In addition, total errors, anticipatory errors, and response patterns, e.g. perseveration of responding to particular positions or objects, the development of response strategies, and the like, can also be examined. To provide flexibility in the testing program, two assistants have been trained to conduct the tests so that the monkeys are used to performing for different experimenters. Details of the training and testing procedures may be found in Bunnell and Perkins (1980).

Training on the task was begun in Mid-January, 1984 and , by mid-June, the three monkeys with previous experience had reached criterion performance on the reversal task. The three inexperienced animals had not yet met the 5-day criterion, but all were having days on which they had three or four errorless reversals on the four problems presented. Tests of the effects of caffeine on performance were conducted 21 - 29 June , and 26 July - 14 August, 1984. Tests of the effects of both caffeine and atropine sulphate were conducted between 6 - 21 September. Baseline testing was interrupted between 17 - 24 August and again from 25 September through 14 October in order to study the effects of interruptions in the schedule upon performance and to determine the time required for retraining to criterion.

Effects of caffeine on WGTA performance. In the first experiment, doses of 12, 4, and 0.8 mg/kg caffeine sodium benzoate were administered im 5 min before testing was begun. (The rationale for the selection of these doses as the initial doses is given in the appendix, which contains the caffeine protocol). Drug days alternated with placebo days (physiological saline) until all animals had received each dose of the drug. (The order of the doses was 12, 0.8, & 4 mg/kg.) Animals were tested 5 days a week, with Monday being a placebo day to account for warmup effects. The second experiment used doses of 24, 16, 8, 4 (twice) and 2 mg/kg with the order being 16, 4, 2, 8, 4, & 24. Doses in the third study were 36 and 4 mg/kg with three monkeys getting one or the other dose on each caffeine day. Doses of 12 mg/kg and higher were scheduled at the end of the week so that 72-96 hours elapsed between the higher doses and the next administration of caffeine. Drug and placebo solutions were coded so that neither the persons administering the injections nor the experimenters doing the testing knew what the animals were getting. Performance on drug

days was compared to average performance across placebo days (Mondays were excluded from this baseline.)

In the first experiment, the 12 mg/kg dose produced an increase in habit formation errors, total errors, or both in all six monkeys. Object quality learning set performance was impaired in only one animal, however. Reversal learning set performance was markedly impaired in three monkeys, but was unaffected or slightly improved in the other three. The 4 mg/kg dose produced mixed results. Total errors were increased in two animals, decreased in one, and unaffected in the other three. Object quality learning set performance was unaffected, but two of the six monkeys exhibited impaired reversals. Interestingly, the number of errors on all reversal trials by the other four monkeys was reduced from a mean of 3.9 on placebo days to 2.3 with the 4 mg/kg dose. At 0.8 mg/kg there was no effect on either object quality or reversal learning set performance, but there was a slight increase in total errors by five of the six animals.

In the second experiment, total errors were reduced substantially by at least one of the doses, although there were considerable individual differences as to which was the most effective dose. The data are shown in Table 4a. There were no consistent effects of any dose on habit formation errors. Although habit formation was impaired in two monkeys at 16 mg/kg, it was improved in one; 24 mg/kg, which was given last in the series, had no effect on this measure in five animals, it increased errors in the sixth case. Trial two performnce on the learning set problems was impaired in two animals with the 16 mg dose, but improved slightly in three animals across a range of doses; one animal's performance was unaffected at any dose. Critical trial performance was impaired at 16 and or 24 mg/kg in three monkeys, but the reversal performance of the other three was not affected.

In the third experiment, the 4 mg dose had no effect on total errors or habit formation errors except in one animal which had 10 habit formation errors (compared to a mean of 4.5 on placebo days) and stopped responding on reversal trials during the third problem. This dose also had no clear effect on either learning set measure. The 36 mg/kg dose had mixed effects on all measures and there were considerable individual differences between animals. Two monkeys had fewer errors and much better learning set performance across the board; three showed slight improvement on object quality learning sets and one was seriously impaired on this part of the task; two were slightly worse on reversal performance and two were much worse. (Table 4b).

When the overall performance is examined across the four administrations of the 4 mg/kg dose of caffeine sodium benzoate, the individual differences in response to the drug are quite apparent. Two animals showed some impairment on the first and or second administration; one had better performance on the first and second, but not later; one was improved on all four administrations, and two were largely unaffected by this dose.

Taken as a whole, the results are generally consistent with the idea (see appendix for a review) that the relationship between caffeine dose and performance is an inverted U-function. It is also clear that the dose response curves are different for different aspects of the tasks being performed. Finally, it seems probable that the drug effects on performance are not associational in nature. Instead, it appears that improvements are due to heightened attention to the test stimuli while the deficits seen at higher doses in some subjects may be due to overarousal which produces competing responses or distraction. It appears that behavioral tolerance developed across experiments in some of the monkeys, complicating data interpretation. Although there is a ceiling effect operating which makes it difficult to detect enhanced learning set performance in some animals, the task appears well suited for uncovering deficits along a number of performance dimensions.

Table 4
Effects of Caffeine Sodium Benzoate on WGTA Performance

a. Total errors and Learning Set Performance Across Dose:

	Dose (mg/kg)						
Animal	24	16	8	4(1st)	4(2nd)	2	SALINE
Errors:							
EASY	8	5	5	5	6	3	7.1
MADISON	6	2	1	6	6	6	8.0
OLIVER	5	4	1	3	2	4	3.9
VULCAN	11	10	2	19	8	7	11.8
SKY	9	8	ILL	ILL	7	6	10.0
YAZTREMSKY	7	12	4	9	3	9	8.6
Correct Reversals out of 4:							
EASY	3	4	3	4	3	3	3.4
MADISON	2	3	4	4	3	2	3.1
OLIVER	4	2	3	3	4	4	3.4
VULCAN	2	1	3	1	4	2	3.4
SKY	3	3	ILL	ILL	3	2	2.6
YAZTREMSKY	4	3	4	4	4	3	3.1

b. Performance under 36 mg/kg caffeine sodium benzoate:

	Habit Errors		Total Errors		Trial 2 Correct/4		Reversal Correct/4	
	Caf	Sal	Caf	Sal	Caf	Sal	Caf	Sal
EASY	3	3.3	10	6.0	4	3.5	2	2.3
MADISON	0	4.3	2	6.3	4	3.0	4	3.3
OLIVER	3	0.5	5	3.0	4	3.8	3	3.5
VULCAN	0	3.5	2	8.8	4	2.8	3	2.3
SKY	1	4.5	10	10.3	4	3.3	1	3.0
**YAZTREMSKY	4**	4.3	4**	7.0	1	3.5	2**	3.0

**Stopped responding on reversal trials after second reversal.

Effects of atropine sulphate on WGTA performance. One experiment was conducted on the effects of doses of .20 and .40 (give twice) mg/kg atropine sulphate on the learning set task using the sam procedures as employed in the caffeine studies. Dosages in this case wer selected on the basis of the pilot studies cited earlier in the section o activity. Dose order was .40, .20, and .40 interspersed with placebo an caffeine trials; atropine trials were separated by a placebo day, caffeine day, and another placebo day. A waiting period of 15 min betwee injection and the beginning of testing was used throughout.

Atropine sulphate disrupted performance at both doses of the drug The data are summarized in Table 5. Performance under .40 mg/kg is give as the mean of t he two administrations of thie dosse. Because there was considerable increase in response failure, errors are given as percent o total responses (exclusive of first trials and reversal trials on eac problem).

Table 5

Effects of Atropine Sulphate on WGTA Performance
Means (+/- S.E.M.)

	Dose (mg/kg)		
Response Measure	.40	.20	SALINE
% Habit Errors	29 (5.00)	28 (4.00)	16 (3.00)
% Total Errors	51 (7.00)	39 (6.00)	19 (3.00)
# No Response	18.5 (4.62)	11.5 (4.49)	0.9 (0.70)
Trial 2 Correct/4	2.3 (0.38)	2.5 (0.50)	3.2 (0.16)
Reversal Correct/4	1.1 (0.29)	2.2 (0.60)	2.9 (0.12)

Significant increases in habit errors, total errors and failures to respond occurred at both doses. When the two administrations of the .40 dose were compared - these were separated by 16 days during which there were several saline and caffeine days as well as the .20 atropine day - there was evidence of sensitization. Mean frequency of failure to respond went from 10.5 +/- 5.18 to 28.0 +/- 5.96; mean habit errors increased from 22% +/- 06% to 38% +/- 05%, and mean total errors from 37% +/- 04% to 65% +/- 01%. Object quality learning set performance, as measured by Trial 2 errors, was moderately impaired at both the .20 and .40 mg/kg doses, but there was considerable variability in individual performance. Similar effects were found for the .20 dose on reversals; only at the .40 dose was there consistent impairment across animals on the reversal problems. In many instances, animals responded correctly on trial 2 and/or the critical trial, even though errors increased substantially on other trials of a problem. In four of the animals, errors began to appear immediately; in the other two, they increased gradually across problems on a given day. Failures to respond increased later in the day's test for

most of the animals that did not respond on all trials. Failures to respond generally occurred first on the reversal phase of the problems and began to affect performance on prereversal trials on later problems. A few monkeys would occasionally refuse to take the raisin reward after making a correct choice and this tended to happen toward the end of the day's problems. The fact that this was comparatively rare suggests that the initial performance decrement is not motivational - this is supported by the activity cage study in which the animals accepted fruit readily 30 - 90 min after similar doses of atropine.

The testing sessions, which began 15 min after drug injection, lasted 25 - 35 min and it is likely that the maximum effects of atropine would occur between 1 and 2 hr post administration. Accordingly, the experiment will be repeated tis fall using a 30 min delay between injections and testing and using doses of .20, .08, and .032 mg/kg. Equivalent doses of atropine methyl nitrate will be given as well as a control for peripheral effects. We suspect that the deficits will be similar for the two forms of atropine.

Effects of interruptions of testing on WGTA. Because the behavioral test battery will require that animals participate in more than one kind of test of performance, we wanted to get some idea of the length of time the animals could go without testing in the WGTA and not have their performance deteriorate. A seven calendar day interruption of testing in August had no effect on object quality learning set performance, but reulted in severe impairment of reversal performance; this recovered with five days of testing. At the end of the atropine study in September, a 24 calendar day interruption produced no deficit on either learning set measure in four animals, slight impairment on reversal sets in one, and severe impairment on both sets in one. After three days, recovery was virtually complete. It appears that performance is relatively resistant to such interruptions and that it will take only a few days to reestablish stable baseline performance. It is possible, however, that interpolating different tasks during interruptions of WGTA testing might cause proactive effects which would alter learning set performance. These six monkeys will begin a free operant avoidance task during the fall after the atropine sulphate/atropine methyl nitrate experiment is completed. During this time, WGTA performance will be sampled at various intervals to study proactive influences. It may be necessary to schedule WGTA retraining trials at fairly frequent intervals to keep the animals at a level of proficiency that will enable them to be brought back to criterion performance rapidly and efficiently.

Relationships between social behavior and WGTA. In an earlier study of the relationships between social status and WGTA performance (Bunnell and Perkins, 1980) we found that high ranking males made more errors on critical trials during reversal learning set training than did low ranking males. In retraining the three oldest monkeys that had previous

experience on the task, the same relationship was observed; however, the relative ranks of these animals were the same as they had been in the initial study, so the significance of this finding is questionable. Nevertheless, the relationship appeared again among the three inexperienced animals during their training on reversals - Yaztremsky, ranked sixth among the males reached criterion first, followed by Sky, ranked fifth, and, finally, Vulcan, the fourth ranked animal. We are in the process of doing a fine-grained analysis of daily social behavior and daily performance on the WGTA task. Preliminary inspection of the data does not reveal any striking relationships between social behavior categories and daily performance, however. There was a spontaneous change in rank in the troop in September; following the first administration of the .40 mg/kg dose of atropine sulphate, Easy, the top ranked monkey, was replaced by Oliver, who had ranked third. Madison dropped to third and the ranks of Vulcan, Sky, and Yaztremsky stayed the same. The resolution of this change in the male dominance hierarchy was completed over the next week to 10 days. During the saline days in this time period, there was no change in either Oliver's or Easy's scores that might be considered to reflect the altered social structure of the troop. Apparently social behavior/performance relationships appear only during acquisition and, once performance has stabilized, the reversal task is insensitive to the influence of social variables.

In the Bunnell and Perkins (1980) experiment cited above, the achievement of stable reversal performance was followed by a stage in which the reversal learning set was extinguished. This was accomplished by giving one reversal trial at the usual place in the problem and then returning to the originally correct stimulus for the remainder of the trials on the problem. The monkey thus had to learn to ignore the reversal cue and continue responding to the previously correct stimulus. Acquisition of criterion performance on the reversal extinction task was related to social rank - once again, high ranking monkeys took longer to reach criterion than low ranking monkeys.

Following the extinction of the reversal learning set, the monkeys require about the same number of trials to relearn the set as they did on original learning. This has the advantage of allowing the study of repeated acquisitions of the set while experimental manipulations of the social status of the animals are performed. However, extinction and reacquisition are very slow and the use of such procedures would not be very practical in a battery of tests developed for screening drugs or for correlating social and performance changes on a day-to-day or even a week-to-week basis. However, it might be valuable to interject extinction problems at intervals during presentation of the normal reversal problems. The effects of such false cues on performance could then be used in the drug studies and might prove to be correlated with social behvaior, particularly during periods of social change. We will evaluate such a procedure during the coming year.

E. Operant Performance:

DRL schedules. The seven oldest males from NT-Troop were trained on a differential reinforcement of low rate of response with a limited hold. The schedule, a DRL-18 sec, LH-10 sec, required the animals to delay 18 seconds between responses before receiving a reinforcement; responding within the 18 seconds reset the timers and instituted another 18 sec delay. The limited hold required that the animal make a response within 10 seconds once the 18 sec delay requirement had been met, otherwise no reinforcer was given. Five of the monkeys had had previous experience on this schedule, the other two did not. By mid June, 4 of the experienced animals had stabilized on the schedule; the two inexperienced monkeys and one old male that had been ill when training began were still on a less stringent DRL-9 Sec, LH-30 sec. During June, and again during August, the effects of caffeine on performance on these schedules was assessed. All animals were then stabilized on the DRL-18, LH-30 sec schedule and tested for the effects of atropine on performance during September.

Animals were allowed to earn 40 reinforcements (banana pellets) during each session; sessions were terminated after 60 min if the animals had not finished. Three measures of performance were obtained: Efficiency Index (EI) - the reciprocal of total responses divided by number of reinforcements obtained. An EI of .50 or larger indicates that the monkey is averaging two or less resonses per reinforcement. (This is generally indicative of highly efficient performance on the DRL schedule. However, when responding drops to a very low rate, such that the limited hold requirement is exceeded repeatedly, EI's may remain relatively high although it takes the monkey considerably longer to obtain its 40 reinforcements.) Response Bursting - the 18 second schedule requirement was divided into six 3 second response bins and bursting was defined as the number of responses in the first bin (interresponse time <IRT> distributions were also obtained, these allow a study of response patterning), and Limited Holds - the number of times the animal exceeded the limited hold requirement during a session.

Effects of caffeine on DRL performance. In the first experiment with caffeine, doses of 12, 4, or 0.8 mg/kg caffeine sodium benzoate were administered im 5 min before the beginning of testing. Placebo (physiological saline) days alternated with caffeine days and Mondays were warmup days during which saline was also given. The results are given in Table 6. (Saline scores are means +/- SEM across four days.) In three of the four monkeys on the DRL-18 sec schedule, the 12 mg/kg dose produced a dramatic increase in total responses that was characterized by bursting during the early part of the delay interval. This resulted in low Efficiency Indexes although all of these animals obtained all 40 reinforcements during the 60 min allowed for the test. The drug produced no consistent changes in the frequency with which the animals exceeded the 10 sec limited hold requirement. The fourth monkey on this schedule requirement had very high baseline reponse rates and

showed only a slight increase in responding with this dose of the drug. This is consistent with the literature which suggests that there is an interaction between the effects of caffeine and baseline operant response rates such that response increases are best observed against a background of low basal rates (see appendix for a review). Similar increases in responding were seen in two of the three monkeys working on the DRL-9 sec schedule; the third animal was not affected by this or either of the lower doses. There was a reduction in the frequency with which two of these monkeys exceeded the 30 sec limited hold requirement, but the effect was small. At the lower doses in the rest of the monkeys, the effects decreased, although four of the animals were still above baseline, even at the 0.8 mg/kg dose.

Table 6

Effects of Caffeine Sodium Benzoate on DRL Performance
Experiment One

a. Efficiency Index:

Animal		DOSE (mg/kg) 12	4	0.8	Saline (./- SEM)
BARKER	#	.24	.32	.43	.53 (.12)
EJU	#	.08	.52	.49	.43 (.10)
HOBBIT	#	.20	.21	.28	.49 (.02)
TAG	#	.12	.11	.12	.13 (.01)
ALLEN	*	.33	.66	.57	.44 (.05)
KUKLA	*	.08	.16	XX	.35 (.02)
WEED	*	.51	.58	.70	.50 (.05)

b. Response Bursting:

BARKER	#	74	44	29	25.5 (12.5)
EJU	#	416	11	6	6.7 (1.9)
HOBBIT	#	90	78	53	23.0 (2.0)
TAG	#	249	282	270	231.5 (10.1)
ALLEN	*	23	8	10	16.8 (4.4)
KUKLA	*	336	176	160	49.8 (6.0)
WEED	*	26	15	12	30.3 (7.3)

c. Limited Hold Exceeded:

BARKER	#	35	44	43	41.8 (17.3)
EJU	#	48	45	35	68.8 (22.7)
HOBBIT	#	55	39	32	39.8 (5.3)
TAG	#	32	45	67	33.3 (3.0)
ALLEN	*	13	17	19	36.3 (8.6)
KUKLA	*	34	34	35	36.0 (8.0)
WEED	*	7	34	23	20.5 (4.6)

DRL-18 sec; LH-10 sec
* DRL- 9 sec; LH-30 sec

From the literature, and from our own pilot observations of animals in the activity cage, we had expected to see a depression of responding at the 12 mg/kg dose, but found an increase instead. We therefore repeated the experiment, using doses of 36, 24, 12, 4 and 0.8 mg/kg caffeine sodium benzoate. Results are given in Table 7; saline scores are means +/- SEM for five days.

Table 7

Effects of Caffeine Sodium Benzoate on DRL Performance
Experiment Two

Animal		DOSE (mg/kg) 36	24	12	4	0.8	Saline (+/- SEM)
a. Efficiency Index:							
BARKER	#	.67	.83	.43	.45	.82	.73 (.03)
EJU	#	.63	.39	.40	.35	.51	.57 (.03)
HOBBIT	#	.10	.12	.16	.47	.45	.48 (.04)
TAG	#	.09	.06	.07	.07	.07	.10 (.006)
ALLEN	*	.40	.49	.41	.68	.71	.67 (.05)
KUKLA	*	.34	.28	.36	XX	.38z	.57 (.05)
WEED	*	.57	.78	.77	.69	.83	.70 (.03)
b. Response Bursting:							
BARKER	#	5	3	25	26	3	6.8 (1.8)
EJU	#	3	16	35	19	5	6.0 (2.2)
HOBBIT	#	215	237	150	26	29	28.0 (4.9)
TAG	#	331	499	503	373	445	296.8 (20.4)
ALLEN	*	21	11	25	9	8	10.4 (2.3)
KUKLA	*	55	77	48	XX	36z	20.8 (5.1)
WEED	*	16	2	9	10	4	10.8 (2.3)
c. Limited Hold Exceeded:							
BARKER	#	25	1	13	17	12	14.0 (4.9)
EJU	#	96	18	35	43	32	36.8 (9.5)
HOBBIT	#	43	31	20	23	61	22.4 (6.6)
TAG	#	18	38	36	81	78	47.0 (4.3)
ALLEN	*	34	13	28	0	30	11.8 (3.7)
KUKLA	*	28	31	20	XX	83z	26.0 (1.3)
WEED	*	3	21	0	0	11	6.0 (2.2)

DRL-18 sec; LH-10 sec
* DRL- 9 sec; LH-30 sec
XX No data - wrong schedule assigned
z Incomplete session - 36 reinforcements

Five of the seven monkeys exhibited significant increases in baseline EI's across the two experiments. Of the other two, one (Hobbit) maintained a baseline EI of approximately .50 in both studies while the other (Tag) continued to respond at a high rate, with a lot of bursting, and did not improve on his low EI. Individual differences in dose response curves were apparent in both experiments, although shifting the curves left or right revealed that the shape of the functions tended to be similar across subjects. The results of the second experiment confirmed those of the first experiment in all major respects and demonstrated the expected drop in responding in several animals at the higher doses. Some monkeys showed better performance at the lower doses, all (even Weed at the 36 mg/kg dose) exhibited lower EIs and increased bursting at one or more doses, and several showed an increase in the frequency with which the limited hold requirement was exceeded at the higher doses, indicating that responding was depressed beyond control levels.

Effects of atropine sulphate on DRL performance. The effects of atropine sulphate on DRL performance were investigated in these same animals in one experiment conducted during September. Doses of .40 mg/kg (given twice to each monkey), .20 mg/kg (also given twice), and .08 mg/kg (given once) were compared to performance averaged across six days on which physiological saline was administered. The order of atropine doses was: .40, .08, .20, .20, and .40 mg/kg. All monkeys were on the DRL-18 sec, LH-10 sec schedule. The results are presented in Table 8. Atropine sulphate produced a substantial disruption of performance at the two higher doses and a somewhat more variable decrement at .08 mg/kg. The animals began testing 15 min after being given the drug; had we waited longer before beginning the test sessions, performance probably would have been even worse, since there is evidence of a progressive failure to respond later in the sessions. The total number of reinforcements received during the sessions is given in part d of the table. Generally, animals that began responding quickly and efficiently under placebo conditions earned more reinforcements under the drug than those that were more dilatory baseline responders. This appears to be due largely to the gradual onset of drug effects as the tests were begun before the maximum effects had been reached.

Although all of the monkeys except Tag routinely earned 40 reinforcements per daily session before the experiment began (Tag averaged about 35), only Hobbit and Weed obtained 40 reinforcements on all six saline days during the experiment, indicating a carryover of drug effects on some placebo days (Table 8d). There were considerable individual differences in the patterning of these carryover effects. One animal, Kukla, consistently failed to earn 40 pellets on placebo days after atropine days; this effect was unrelated to dose. Eju missed between 1-9 pellets on saline days following the first four drug days, but received 40 after the second administration of .40 mg/kg. Two animals were affected early in the experiment: Tag received only three pellets on the day after the first .40

mg/kg dose, but earned 40 on all other saline days while Barker was affected by the first doses of .40 and .20 mg/kg but not by .08 or the second administrations of the two higher doses. Allen failed to complete the session on the days following the .08 and the second .40 mg/kg doses, but earned 40 pellets on the other saline days.

Under atropine sulphate, EI's generally declined, bursting declined, the frequency with which limited holds were exceeded increased, and total reinforcers received dropped - markedly in most cases. The few cases where EI's improved or were unchanged may be accounted for by the decreases in bursting and declines in overall responding. Two interesting exceptions to the general changes in response patterns were seen. Hobbit, who is a fast, efficient responder under baseline conditions, exhibited a significant increase in bursting at all doses of atropine, a significant increase in LH's exceeded on 4 of the 5 days, and an across the board decrease in EI. Kukla, a less efficient baseline performer than Hobbit, showed a similar pattern or some, but not all, atropine days. Barker, at the first administration of each dose, and Weed, at the .08 mg/kg dose, also exhibited increased bursting. There appear to be some interesting changes in performance taking place before all responding is suppressed by atropine. The nature of these changes is not clear from the present experiment. To examine the problem further, and to check for the relative importance of peripheral vs central effects of the drug, an additional experiment is scheduled for the winter of 1985. Doses of .20, .08, and .032 mg/kg of both atropine sulphate and atropine methyl nitrate will be used in conjunction with delays between drug administration and the beginning of testing of 5 min, 15 min, and 30 min. The addition of the lower dose, and of the shorter and longer drug administration/test intervals, will enable us to examine the course of performance changes across time; at certain doses and delays, we would expect to see similar patterns across individual animals.

Based on our initial study of atropine effects on activity and ingestive behavior with the C-Troop animals, we do not think it likely that the primary effects of atropine on DRL performance are the result of the drug induced thirst which causes the monkeys to stop responding for the banana pellets. However, we will make water available in the operant chambers on some days (we have to avoid inducing adjunctive drinking) and will monitor food and water consumption during the period immediately after the monkeys are removed from the test boxes and before they are returned to their social group.

In the initial experiment, some tolerance appeared to develop; however, the confounding of dose and order of administration of the different doses in conjunction with individual differences in response to the drug makes this difficult to assess. It may be necessary to schedule a third experiment to examine tolerance effects at a later date.

Table 8

Effects of Atropine Sulphate on DRL Performance
(DRL-18 sec; LH-10 sec)

Animal	Dose (mg/kg) .40(1)	.40(2)	.20(1)	.20(2)	.08	Saline (+/- SEM)
a. Efficiency Index:						
Barker	.20	.25	.16	.30	.26	.59 (.08)
Eju	.09	.33	.30	.67	.80	.60 (.03)
Hobbit	.12	.22	.17	.23	.28	.50 (.03)
Tag	.12	.14	.13	.13	.06	.18 (.05)
Allen	.26	.33	.41	.38	.33	.37 (.06)
Kukla	.11	.09	.15	.07	.07	.24 (.03)
Weed	.21	.42	.29	.30	.22	.35 (.03)
b. Response Bursting:						
Barker	23	3	15	5	12	8.7 (1.6)
Eju	0	2	2	1	0	4.8 (1.2)
Hobbit	148	82	99	89	64	24.1 (1.8)
Tag	118	69	143	87	124	209.4 (24.3)
Allen	7	2	6	7	9	17.3 (5.9)
Kukla	126	80	26	88	81	52.0 (11.0)
Weed	24	11	23	34	71	41.2 (6.6)
c. Limited Hold Exceeded:						
Barker	134	168	142	121	152	55.0 (26.6)
Eju	142	167	147	127	166	80.0 (20.6)
Hobbit	82	32	91	63	69	30.0 (0.9)
Tag	118	37	117	99	152	64.5 (21.3)
Allen	131	168	107	79	116	68.8 (19.0)
Kukla	79	142	136	99	147	105.7 (13.5)
Weed	119	100	100	92	82	28.0 (5.7)
d. Total Reinforcers Received:						
Barker	11	2	3	5	13	30.4 (6.2)
Eju	4	3	3	2	4	37.5 (1.4)
Hobbit	37	40	36	40	40	40.0 (0.0)
Tag	19	12	19	17	10	33.8 (6.2)
Allen	11	2	32	29	34	31.3 (6.2)
Kukla	25	11	8	9	9	25.3 (3.8)
Weed	15	22	23	25	39	40.0 (0.00

DRL performance and social behavior. In our previous work (Bunnell, 1982) we had found two relationships between DRL performance and social variables. During initial training on the schedule, the achievement of efficient performance on the schedule was positively correlated with high social rank. Once performance stabilized, response bursting was positively correlated with the frequency of aggressive responses exhibited by each monkey. This was demonstrated experimentally by removing and replacing animals of varying social rank in the groups and relating changes in aggressive response frequency produced by these manipulations to changes in DRL performance.

Five of the monkeys in the present experiments had participated in the earlier study and one of these, Weed, began retraining late because of illness. In June, the other four animals were performing well on the DRL-18 sec schedule while Weed and the two inexperienced animals were still on the less stringent DRL-10 sec schedule. Because of differences in stage of training, the efficiency ratios of all seven animals cannot be compared directly. However, within each subset of animals, the highest ranking ranking animals had the best efficiency ratios (ERs) within their groups:

DRL-18 sec			DRL-10 sec		
Animal	Rank	ER	Animal	Rank	ER
Barker	1	.53	Weed	3	.50
Eju	2	.43	Allen	5	.44
Tag	4	.13	Kukla	6.5	.35
Hobbit	6.5	.49			

After all seven animals were on a DRL-18 sec schedule, the rank order correlation between ER and social rank was +.63 at the time the atropine sulphate study described above was run. This is despite the fact that three of the animals had not reached maximum efficiency at this time and that the ER data were taken from scores on placebo days when there may have been some carryover of drug effects. Despite the presence of several confounding factors, it is encouraging that the same trend toward a relationship between social rank and efficient performance we had seen in the earlier work also appears in the current data. However, relationships seen during training and retraining, although interesting, will not be of much practical use in a battery of tests developed for assessing performance changes related to social variables and drug effects.

Although Tag had the highest frequency of aggressive responses and showed the most response bursting during the placebo days of the atropine experiment, the overall correlation between between frequency of aggressive behavior and bursting was low. The appearance of a relationship between bursting and aggression in the initial studies took place during and immediately after manipulations of the social structure of the troop by removal and replacement of males. This experiment is now being conducted; Barker, the alpha male, was removed from the troop during the last week of September and will be reintroduced in October. This manipulation has produced an increase in fighting among the other males and it appears likely that one or more rank changes are imminent. Data obtained during the next few weeks with Barker out and

following his return will give us an excellent test for the presence of the expected relationship between aggression and performace.

Fixed Interval schedules. Performance on fixed interval (FI) schedules, in which the animal receives a reinforcer for a response made after a preestablished time interval has passed, has been shown to be sensitive to caffeine (see the appendix for a review). For this reason, and because we have previously seen a relationship between performance on this schedule and social variables in rhesus monkeys (Bunnell, et al, 1979a,b), the I-Troop males were placed on a FI schedule and the effects of caffeine sodium benzoate and atropine sulphate on performance examined. The eight I-Troop males began training at the end of March 1984. Training was very slow and, by mid-June, the majority of the animals had progressed only to a FI-20 sec schedule; although they were completing their sessions, they showed little evidence of the response curve scalloping which indicates temporal discrimination and efficient performance on the task. By mid-August 6 of the animals were shifted to a FI-30 sec schedule and a seventh was put on this schedule two weeks later. The eighth monkey, Alabama, who ranked second in the social group, was not performing consistently and is excluded from the data presented below. By mid-September, seven animals were showing good scalloping in their response curves - the 30 sec interval was divided into six 5-sec bins and the animals had positive indexes of curvature (IC's) indicating that the majority of the responses were occurring in the last 15 sec of the 30 sec interval. The other dependent measures were the frequency of responses in the first 5-sec bin following a reinforcer - a measure of response bursting, and the ratio of responses to reinforcements - a measure of overall response rate.

Effects of caffeine on FI performance. A pilot experiment, conducted in June during training and before performance had stabilized, utilized doses of 12, 4, and 0.8 mg/kg caffeine sodium benzoate alternated with placebo (physiological saline) days. The daily sessions were started 5 min after the im injections of the drug. At the 12 mg/kg dose, response rates increased in three monkeys, decreased in three, and were unchanged in one. There were no obvious effects at the two lower doses. In the main experiment, conducted in conjunction with an experiment on atropine effects, doses of 36, 12, and 4 mg caffeine sodium benzoate were administered 5 min before begining testing on a FI-30 sec schedule. The results for each animal are shown in Table 9. Saline scores are the means +/-SEM for 12 placebo days during the experiment. The dependent measures were the presence or absence of scalloping (presence of a positive IC), number of first bin responses (a measure of bursting), mean responses per reinforcement, and total number of reinforcers received.

Table 9
Effects of Caffeine Sodium Benzoate on FI Responding (FI-30 sec)

Animal	Dose(mg/kg) 36	12	4	Saline (+/- SEM)
a. Scalloping:				
Cracker	NO	NO	YES	YES 11/12
Equal	NO	NO	NO	YES 7/12
Gus	NO	ILL	YES	YES 7/12
Quotation	NO	YES	YES	YES 12/12
Spiro	NO	NO	YES	YES 11/12
Yamamoto	NO	YES	YES	YES 11/12
Yuk	NO	YES	YES	YES 12/12
b. First Bin Responses:				
Cracker	29	42	14	14.5 (1.6)
Equal	117	136	76	72.3 (5.9)
Gus	36	ILL	57	58.7 (8.3)
Quotation	2	6	3	14.5 (6.0)
Spiro	37	21	16	24.6 (3.1)
Yamamoto	60	87	50	35.3 (3.5)
Yuk	36	4	5	6.5 (2.1)
c. Responses/Reinforcement:				
Cracker	2.5	3.5	2.0	2.2 (0.9)
Equal	5.8	7.6	3.5	3.8 (0.3)
Gus	4.5	ILL	4.8	4.3 (0.3)
Quotation	2.3	1.5	1.7	1.8 (0.2)
Spiro	4.1	2.4	2.0	2.3 (0.2)
Yamamoto	3.5	4.6	3.1	2.5 (0.2)
Yuk	2.7	1.4	1.7	1.7 (0.1)
d. Reinforcements Received:				
Cracker	40	40	40	40.0 (0.0)
Equal	36	32	40	38.3 (0.9)
Gus	21	ILL	30	34.2 (1.7)
Quotation	3	22	37	39.5 (0.5)
Spiro	40	40	40	40.0 (0.0)
Yamamoto	40	40	40	40.0 (0.0)
Yuk	40	40	40	40.0 (0.0)

As in the other caffeine studies being reported here, there were considerable individual differences in the effects of the different doses. The most consistent effect was the loss of response scalloping in all seven monkeys at the 36 mg/kg dose; scalloping was present in half of the animals at 12 mg/kg and in six at 4 mg/kg). This was accompanied by increased first bin responses in five animals at 36 and/or 12 mg/kg. In three

cases, increases in reponding during the first bin were greater at the 12 mg dose than at 36 mg/kg, suggesting the presence of the inverted U-shaped dose response relationship discussed earlier; however, since the larger dose was given last, these changes might be tolerance effects. Most animals showed an increase in the response/reinforcement ratio at one or more dose levels. The results are consistent with data from other laboratories in that increases in response rate occur on this schedule at some doses of caffeine. However, the decreased temporal discrimination evidenced by the loss of scalloping has not been observed in the other studies (see Appendix, pp 9-10 for a review of caffeine effects on FI performance.) As caffeine was being used here primarily as a positive control drug for the comparison of atropine effects, no additional work on caffeine effects on this schedule is contemplated in the near future, and the animals will be shifted to a new schedule once the study of atropine effects on FI performance described in the next section has been completed.

Effects of atropine on FI responding. This first part of this experiment was done in association with the study of caffeine effects described in the preceding section. There were two administrations of .20 mg/kg atropine sulphate, one with 15 min and one with 60 min between drug administration and the beginning of testing, and three adminstrations of .08 mg/kg, one with a 15 min and two with a 60 min delay. The order of administration was: .20 - 15 min delay; .08 - 15 min delay; .20 - 60 min delay; .08 - 60 min delay; .08 - 60 min delay.

The .20 mg/kg dose caused the animals to respond slowly and to not finish the test sessions; in comparison with the 15 min delay, the 60 min delay produced marginally poorer scores in terms of number of pellets earned (Table 10d), but the animals were still making some responses. The appearance of scalloping in three animals (10a) with the 60 min delay probably is the result of a drop in overall responding rather than a reestablishment of temporal discrimination. With one exception, first bin responding was depressed at both delay intervals (10b) and this was associated with a decline in overall response rate also since scalloping was impaired. Despite the overall decline in responding, responses per reinforcement tended to increase in six of the monkeys at at least one delay interval (10c). The animals appeared to have lost the ability to make a single, discrete response when they did respond.

At the 15 min delay, there was little effect of the .08 mg/kg dose on the six monkeys tested and all completed the session and obtained 40 banana pellets. However, three exhibited impaired scalloping while two exhibited increases and three decreases in first bin responses; the animal that showed the most bursting also increased its responses/reinforcement. With the first 60 min delay at the .08 mg/kg dose, scalloping was present in 6 of 7 monkeys, but was lost in five of these animals with the second administration. Increased response bursting, accompanied by a trend toward increased responses/reinforcement, was seen in five monkeys on either the first or second administration of this dose with the 60 min

delay. However, there were also substantial decreases in first bin responding on one or more occasions in several of these animals. At this dose ano delay interval, two animals failed to complete the session on the first administration and five failed on the second administration. This, and the changing patterns of first bin responses suggest the possibility of behavioral sensitization to the drug.

The experiment is continuing with the addition of a .032 mg/kg dose and the use of a 30 min delay between injection and testing with both .20 and .032 mg/kg doses. Results will be available in the January, 1985 quarterly report.

FI performance and social behavior. In our earlier work (Bunnell, et al, 1979a, 1979b and Bunnell, 1982) we had found relationships between bursting on FI schedules and social rank in rhesus monkeys but not in M. fascicularis. The data from I-Troop for the month of September for bursting and responses per reinforcement yielded very low correlations with social rank during that time. There were very few agonisitic behaviors in the troop during this period and there was no suggestion of a relationship between social behavior and performance:

Animal	Rank	1st Bin Resp	Resp/Reinf
Gus	1	58.7	4.6
Spiro	3	24.6	2.5
Yamamoto	4	35.3	2.5
Cracker	5	14.5	2.2
Yuk	6	6.5	1.6
Quotation	7	14.5	1.8
Equal	8	72.3	3.8
		rho = +.15	rho = +.42

It is of interest to note that the corrrelations would have been +.67 and +.79, had Equal's data been excluded. A similar, but less severe impact on the correlation between rank and performance is seen in Hobbit's ER data in NT-Troop (see section on DRL performancce and social behavior). Perhaps there is something about very low ranking males that produces consistently different performance. This possibility will be examined very carefully in the coming months. Since the FI schedule was used primarily to look at caffeine effects and these aniamals are being shifted to a VI schedule very soon, we have no plans for additional studies of social behavior and FI performance at the present time.

Table 10
Effects of Atropine Sulphate on FI Responding (FI-30 sec)

	Dose					
	.20 mg/kg		.08 mg/kg			Saline (+/- SEM)
Delay (min):	15	60	15	60(1)	60(2)	
Animal						
a. Scalloping:						
Cracker	NO	NO	YES	YES	NO	YES 11/12
Equal	NO	YES	NO	YES	NO	YES 7/12
Gus	NO	NO	-ILL-	YES	NO	YES 7/12
Quotation	NO	NO	YES	YES	NO	YES 12/12
Spiro	NO	NO	NO	YES	NO	YES 11/12
Yamamoto	NO	YES	NO	NO	YES	YES 11/12
Yuk	NO	YES	YES	YES	YES	YES 12/12
b. First Bin Responses:						
Cracker	7	6	16	11	13	14.5 (1.6)
Equal	59	29	126	53	93	72.3 (5.9)
Gus	1	8	-ILL-	131	36	58.7 (8.3)
Quotation	2	0	2	3	16	14.5 (6.0)
Spiro	35	23	36	21	46	24.6 (3.1)
Yamamoto	9	8	28	366	59	35.3 (3.5)
Yuk	4	1	1	3	25	6.5 (2.1)
c. Responses/Reinforcement:						
Cracker	1.9	2.4	2.1	2.1	2.2	2.2 (0.9)
Equal	5.5	2.8	5.9	2.6	4.4	3.8 (0.3)
Gus	1.5	2.9	-ILL-	11.8	3.8	4.6 (0.3)
Quotation	1.5	3.0	1.6	1.9	2.6	1.8 (0.2)
Spiro	3.6	3.9	2.3	2.7	3.9	2.5 (0.2)
Yamamoto	2.9	4.0	2.1	11.7	6.0	2.5 (0.2)
Yuk	1.6	2.3	1.2	1.6	2.2	1.6 (0.1)
d. Reinforcements Received						
Cracker	27	20	40	40	32	40.0 (0.0)
Equal	21	20	40	40	40	38.3 (0.9)
Gus	2	7	-ILL-	23	20	34.2 (1.7)
Quotation	8	2	40	31	11	39.5 (0.5)
Spiro	25	23	40	40	29	40.0 (0.0)
Yamamoto	8	4	40	40	20	40.0 (0.0)
Yuk	30	7	40	40	40	40.0 (0.0)

H. Equipment and Facilities:

A battery of six individual cages was obtained and set up in a separate colony room to house the C-Troop males. A 2 x 2 x 2 m cage was fabricated locally and installed in the inner room of the two room suite used for the observation and testing of the C-Troop males. The outer room, from which the observations are made, also houses programming equipment for an operant conditioning chamber used in the pilot work on the free operant avoidance task. This chamber was made available to the project by the Department of Pharmacology of the College of Pharmacy and has been set up in a separate testing room. Eight new constant current shockers were purchased for use in the free operant avoidance task, and components for two primate test panels to be used in the tests of cooperative behavior in C-Troop were received. Four single board computers were ordered for use in controlling the cooperative task and for running operant schedules in the animal compounds. (These were out of production when we first tried to order them, but have since become available again.) A new interface for use in controlling operant testing with the PDP-8 laboratory computer was purchased.

During the first few months of the contract, the open field was repainted and all of the operant chambers and their associated manipulanda, inputs, pellet dispensers, and the like were repaired and refurbished. The floor of the colony room containing the indoor cages for T-, NT-, and I Troops, which had been specially treated and covered a few years ago, began to peel in spots. A request for estimates for removing the existing covering and refinishing the floor was submitted in July and the University of Georia Research Foundation has committed the funds to make these repairs which are supposed to be completed before cold weather arrives this fall. The refinished floor will make sanitation of the indoor quarters much easier and will be a help in maintaining the colony in good health.

During the year, problems were experienced with the PDP-8E laboratory computer used to run the operant programs and to analyze the social data. A combination of an aging and increasingly unreliable machine with the inability of the manufacturer to provide timely and competent maintenance has been a constant source of delay and irritation. A proposal to purchase a new system, based upon a PDP-11 computer, together with the associated interface to run the SKED-11 operating system, was submitted to the Command in March. The proposal was approved and funds became available in September. It is expected that the new system will become operational during the winter of 1985. The new system will also take the place of the small Commodore system requested in the original contract proposal. This system was to have been employed as a mother computer for controlling the single board computers to be used with the cooperative task and the operant testing in the outdoor compounds. Funds originally budgeted for the system are being diverted to the PDP-11 system.

I. Personnel:

Dr. Bunnell, of the Department of Psychology, serves as principal investigator for the project. Dr. Iturrian, from the Department of Pharmacology and Toxicology, is coprincipal investigator. The consulting veterinarian is Dr. Willy L. Chapman, Jr. from the Department of Pathology of the College of Veterinary Medicine at the University of Georgia. Additional veterinary care and support are provided as needed by the staff of the veterinary college and by the university's Animal Care Coordinator from the Office of the Vice President for Research. A full time animal caretaker, who has a batchelors degree in biology, was hired in November, 1983. A full time research technician, with electronic and computer interfacing skills, began work in February, 1984. This individual has a batchelors degree in psychology and has served as an electronics technician in the U. S. Army. He is responsible for supervising the laboratory schedule in addition to his duties as a technician. He is also enrolled as a part time graduate student in psychology at the University of Georgia and has been assigned the development of the operant cooperative behavior task as a Masters thesis project. Two part time graduate research assistants have worked on the project from the start. Both of these people have masters degrees in psychology. One is specializing in primatology in his doctoral work. His work on the project involves observing social behavior and testing the animals on the WGTA tasks. The second, a doctoral candidate in physiological psychology, does social testing, testing in the open field, and assists the research technician with the operant testing. A third graduate assistant is a premasters student in physiological psychology. He was hired in July to replace an undergraduate assistant who had been helping with the WGTA testing and the social observations. His primary responsibilities are in WGTA testing and in running the activity and social tests with the C-Troop males. The animal caretaker, in addition to her caretaking duties, does social testing, open field testing, and maintains the animal colony records.

Summary and Future Work

Much of the early part of the contract year was devoted to training personnel, adapting the animals to the testing routines, and equipment and facility repair and improvements. Behavioral testing in the laboratory, begun in the winter, and social testing, begun in the spring, resulted in the acquisition of considerable baseline data on two operant schedules, complex problem solving, open field behavior, and social behavior in the four troops of monkeys. Testing of the effects of caffeine on these behaviors was essentially completed, and tests of atropine were well underway by the end of September.

Social data gathered on T-, NT-, and I-Troops make it clear that both group scan and focal animal observation procedures need to be employed in the project; the former for isolating drug effects and the latter for maximizing the

detection of social behavior/performance relationships. The extensive data gathered during the spring, summer, and fall will be analyzed during the winter of 1985 to determine the best combination of the procedures for meeting the projectobjectives. Caffeine had no effect on social behavior; studies of the effects of atropine are currently in progress. Tests of dyadic interactions with the C-Troop males yielded little usuable data because agonistic interactions were relatively infrequent. We plan to introduce unfamiliar animals in an effort to increase agonistic behavior. To study cooperative behavior in a social setting, "enlisting" behavior will be studied during the coming months using the C-Troop males.

Tests of open field behavior proved sensitive to drug effects, but relationships between open field exploration and social variables were ambiguous. An attempt to verify the finding of a relationship between social rank and frequency of contacts with novel objects will be made once a planned study of the effects of atropine sulphate vs atropine methyl nitrate is completed. A study of the effectiveness of using social stimuli (strange monkeys) in the open field is also planned for the coming year.

Speed of acquisition of learning sets is negatively correlated with high social rank. Performance on these tasks is very sensitive to drug effects and the tests yield a variety of measures of different aspects of performance. However, once learning set performance has stabilized, the relationships between social variables and performance largely disappear. During the coming year, the relationship between reversal extinction probes and social variables will be examined.

Both DRL and FI schedules are sensitive to caffeine and atropine effects. Acquisition of efficient DRL performance was positively correlated with social rank, but an expected correlation between response bursting and aggressive behavior did not appear. A study in which the social structure of the group is being manipulated to increase aggression is under way. No further work with the FI schedule, which was used to verify caffeine effects on operant performance, is planned.

Drug testing during the coming year will involve completing tests of the effects of atropine sulphate and atropine methyl nitrate on the tasks on which the animals have been working. When training on the new tasks is completed, tests of the effects of caffeine, the atropines, diazepam, and, time permitting, pyridostigmine will be conducted with them.

During the coming year, we will train the T-Troop males on a free operant avoidance task in addition to continuing the evaluation of the WGTA procedures described above. NT-Troop males will be tested on fixed ratio schedules while living in their social group. I-Troop animals will be trained on a VI schedule and then given MULT VI - VI and VI - EXT schedules. C-Troop males will begin shaping on operant performance with the objective of developing a two-animal task that will require "cooperation" between monkeys. C-Troop, as noted above, will also be involved in the attempt to bring enlisting behavior under experimental control in order that we may study a more natural type of social cooperation.

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APPENDIX

Effects of Caffeine on Social Behavior and Performance

RESEARCH PROTOCOL
Effects of Caffeine on Social Behavior and Performance

(To be conducted under Contract Number DAMD17-83-C-3260: The Effects of CW-Related Chemicals on Social Behavior and Performance.)

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Purpose:

This protocol is submitted in accordance with the provisions of the research contract named above. It describes the procedures that are to be used in studying the effects of caffeine on social behavior and performance in non-human primates. The two drugs whose effects on social behavior and performance are to be studied during the first year of the contract are atropine and caffeine. A protocol for atropine was submitted and approved at the time the original research proposal was approved. The protocol for caffeine parallels the atropine protocol and, does not repeat all of the behavioral testing procedures described in the earlier document. In the caffeine protocol, we provide a brief background of the pharmacology and behavioral effects of caffeine, describe the general methods and procedures to be utilized, and, where appropriate, provide a statement of the expected results. As each step in the behavioral testing procedure is completed for caffeine, we will then test the animals under atropine. Experience gained from using the behavioral testing schedule in the caffeine studies will be incorporated into the procedures to be used in testing atropine effects.

Background:

Pharmacology of caffeine.

Caffeine is to be used as a positive control drug for the stimulant effects of atropine, which will be studied in parallel with it. The diverse pharmacological actions of caffeine once found many medical uses. Today, except for "over the counter" preparations, it has few accepted therapeutic uses (Goldman, 1984) and is primarily a dietary habit that may contribute to medical presenting symptoms (Rall, 1980). However, since it interacts with other drugs, it may alter the response to agents that might be used as CW antidotes or prophylactics.

Caffeine is the most widely used social stimulant in our society as there is a general assumption that in moderate amounts it presents little risk of harmful effects. Recent research has called this

assumption into question by demonstrating a complicated array of biochemical, physiological, and behavioral effects. Recent reviews of caffeine's pharmacological actions (Rall, 1980; Lachance, 1982; Katmis, Murphy and Snyder, 1983), biochemical effects (Kuczenski, 1983), behavioral effects (Sawyer, Julia and Turin, 1982), toxicity (Lachance, 1982) and methodological issues (Grossman, 1984) are available. Much of the enormous amount of current research on caffeine consists of studies of genetic (mutagenicity and teratogenicity) or enzymatic effects in isolated cells. However, the search for effects on endurance, learning and performance has continued since the first behavioral pharmacology paper was published by Zavadskii (1908) early in the century.

Almost all organ systems are influenced by caffeine, although the most prominent effects involve the stimulation of the CNS and cardiac and skeletal muscle; this is accompanied by a relaxation of bronchial and other smooth muscle. The adverse effects of average doses include diuresis, restlessness, unsteadiness, and gastric secretion. Emesis, tachycardia, arrhythmias, tinnitus, and tonic-clonic convulsions occur with large doses, but death is rare.

Specific Considerations. Caffeine is an undissociated weak electrolyte at physiological pH with limited solubility (1 gm/46 ml water), although it is ten times more soluble in boiling water. Solubility is increased by hydrobromide, hydrochloride, phosphate, or salicylate, but these salts, even in a nonaqueous medium (ethanol and PEG) are quite acidic and readily decompose. Caffeine citrate is the soluble form utilized in soft beverages, but it is not suitable for hyopodermic administration because of its acidity. The bitter taste of caffeine is difficult to mask and some studies which used peroral cafeine found their subjects (rats or monkeys) sometimes refused the drug even when it was given in fruit juice. After oral intake, the absorption of caffeine varies widely between individuals of all species (Goldman , 1984). Furthermore, since the reference drugs for this program will be administered parenterally, the commonly used oral route becomes less appropriate for the caffeine studies covered by this protocol.

A mixture of equal parts caffeine and sodium benzoate forms a complex double salt that is slightly alkaline (pH about 8) soluble solution (1 g/1.1 ml water) suitable for iv, im, or sc injection. Although rarely used now, caffeine sodium benzoate - parenteral injection - has been used as a cardiac, respiratory, and psychic stimulant to treat barbiturate and morphine overdose. Benzoate is widely used as a preservative in drugs and foods (limited to 0.1% and a maximal acceptable intake of 5 mg/kg/day). Although benzoate will not be troublesome in this study, it is not inert - it displaces bilrubin from binding, an effect that has been used as a test for liver function. Aceto, Carchman, Harris and Flora (1978) found 32 mg/kg ip elicited restless pacing, tremors, and vocalization in morphine dependent rhesus monkeys. Similar effects were seen with 4 mg/kg caffeine, a result to be expected since caffeine is 10 times more active than sodium benzoate in inhibiting cAMP phosphodiesterase activity. (It may be noted that the parenteral formulation for another xanthine, theophylline (1,3 dimethyl xanthine), which utilizes ethylene diamine to make aminophylline, would not produce a

soluble salt of caffeine (1,3,7 trimethylxanthine) as the latter lacks an acidic site.)

Once absorbed, caffeine enters the intracellular water of all tissues almost equally and there is no blood brain barrier to caffeine (Burg and Werner, 1972). Plasma concentration curves following oral and intravenous dosage are superimposable, suggesting that there is not much of a first pass - liver metabolism effect. Only minimal amounts of caffeine are excreted unchanged in any of the species studied, but metabolic products vary widely between species (Lachance, 1982).

The plasma half life (t1/2) of caffeine varies depending on the age, sex, species, and behavioral history of the test animals. It is reported to be more toxic in the young and in males as opposed to females. Primates are much more sensitive than rodents (Lachance, 1982). The t1/2 in dogs is 5 hr (4 mg/kg) and man has a t1/2 of about 4.5 hr (7 mg/kg), but this ranges from 2.5 hr (for smokers) to 6 hrs as metabolism is the rate limiting factor in caffeine's clearance from the plasma (Parsons and Neims, 1978). The half life in pregnant baboons (4.4 mg/kg) was about 11 hours, but gravid females metabolize caffeine very slowly (Christensen, Kling, and Manion, 1979). Elimination from saliva, plasma, and amniotic fluid had similar half lives for these baboons. The elimination kinetics is probably dose dependent as the half lives averaged 0.6 to 1.7 hours in mice following doses of 5 and 25 mg/kg respectively (Burg and Werner, 1972).

Many of the effects of caffeine are biphasic (Lachance, 1982). For example, bradycardia is the predominant response of humans to small doses (50-100 mg) of caffeine, but large doses (200-500 mg) result in tachycardia. In rodents, caffeine alters locomotor activity with a complex, triphasic dose response curve. In mice, very low doses of caffeine (1-2 mg/kg) depress activity while 10-30 mg/kg produce a marked increase in activity (Katims, et al, 1983); however, at 50 mg/kg, activity declines to basal levels. A 100 mg/kg dose inhibits locomotor activity by 90%, while 175 mg/kg induces a loss of righting and convulsions occur at 200 mg/kg (Seale, Johnson, Carney & Rennert, 1984). Similar biphasic dose response profiles also occur in rats (Kuczenski, 1983). Davis, Kensler & Dews (1973) found modest increases in the physical activity of squirrel monkeys at 3 mg/kg, but 10 and 30 mg/kg depressed activity.

The various effects of xanthines are differentially altered by benzodiazepines, amphetamines, neuroleptics and adenosine analogs (Kuczenski, 1983). Therefore it is possible that the stimulant, the depressant, and the convulsant effects might be mediated by different neurochemical mechanisms. Although attractive, this idea has not been substantiated as yet.

The benzodiazepine antagonist Ro15-1788 blocks the convulsive (Vellucci and Webster, 1984), but not the locomotor effects of caffeine (Katims, et al, 1983). The locomotor effects exerted by caffeine appear to involve blockade of adenosine receptors (Snyder, et al, 1981), but dopaminergic effects are also evident (Anden and Jackson, 1975; Waldeck, 1975).

Behaviorally inactive doses (0.3-0.6 mg/kg) of caffeine abolish the locomotor and anticonflict effects of diazepam in rats and mice. Three to 10 mg/kg of caffeine reduce the muscle relaxant (Polc, et

al, 1981) and drug discrimination effects of diazepam in primates (Griffiths, 1982) whereas 50 mg/kg are necessary to suppress antiaggressive effects in cats. Very high doses (160 mg/kg) are required to antagonize the anticonvulsant action of diazepam (Velucci and Webster, 1984) or the respiratory depression produced by barbiturates or morphine (Rall, 1980).

Caffeine elicited locomotor stimulation is quite different from that produced by amphetamine (Foy, 1969; Hughes and Grieg, 1976). When caffeine (7.5 mg/kg) precedes d-amphetamine by less than an hour there is an additive effect on locomotor responses; however, pretreating the animals 12 hours ahead attentuates the amphetamine effect (White and Keller, 1984). Caffeine has been reported to sensitize catecholamine receptors (Waldek, 1975) while this last effect represents tolerance.

Whether any of these drug interactions reflect a direct linkage of benzodiazepine receptors and adenosine systems is unclear (Katims, et al, 1983) as is the role of the catecholamines.

Tolerance. There is marked interindividual variation in behavioral response to caffeine in man (Goldstein, Kaiser, and Whitley, 1969), mice (Seale, et al, 1984) and, apparently, in nonhuman primates (Aceto, et al, 1979). The somatic manifestations involving the CNS, cardiovascular, gastrointestinal system and the diuretic effects are cognitively identified by high and low consumption subgroups (Goldstein, et al, 1969). Some individuals were very senstivie to caffeine induced gastrorrhea and the CNS effects. There are also major individual differences in absorption and biotransformation. The relative importance of genetic and environmental factors in these individual differences has not been established (Seale, et al, 1984).

Tolerance will develop to the diuretic, cardiovascular, and CNS effects of caffeine over a period of time (Rall, 1980). However, the effects are of low magnitude as doubling the usual chronic dosage consumed will restore the full response (Colton, Gosselin and Smith, 1968). Caffeine will increase enzymatic activity of the microsomal drug metabolism system, but stopping the drug for 24 hours nullifies this induction (Mitoma, Lombrozo, LeValley and Dehn, 1969). In rats, 30 to 60 mg/kg, administered at 12 hour intervals, were required to produce tolerance to the locomotor activity effects of caffeine (White and Keller, 1984). Caffeine sensitizes the dopamine receptor, but the effect persists for about an hour (Kuczenski, 1983). Kindling requires a large dosage over many days.

The majority of the effects on catecholamines and stress hormones that are induced by caffeine involve chronic dosage (Henry and Stevens, 1980) and therefore are the result of a combination of acute sensitization and tolerance. From the literature that has been reviewed, it would appear that neither tolerance nor sensitization will be a problem for the studies in this protocol if moderate dosage is given on alternate days.

Dosage. Drug dosage and time courses to be used will be determined empirically from our animals' behavioral response to initial doses selected from the literature. These doses will be chosen to have comparable effects both to human dietary habits (e.g., Burg, 1977, estimated that the average American adult's caffeine

intake was 2.4 mg/kg/day,) and to pharmacological and behaviorally relevant doses in other species as well.

Comparing differences in drug responsiveness in different species or individuals on the basis of equal plasma levels with comparable serum half lives usually produces more similar levels of responsiveness than when comparisons are made on the milligram per kilogram of body weight basis (Koppani and Avery, 1966). Serum concentrations might be useful in explaining unanticipated individual differences in the monkey's responses. There are facile, sensitive HPLC assays for caffeine (Simons, Frith, and Simons, 1982) should a measure of t1/2 appear useful in our studies.

Although very recently caffeine has become a popular research tool for the pharmacologist (Grossman, 1984), the biphasic effects and the possibility of multiple receptor actions dictate caution in dosage selection. There are a few primate studies that may be helpful in selecting an initial dose. Deneau, Yangita, and Seevers (1969) reported no toxicity with 1, 2.5, and 4 mg/kg (iv), but self administration was sporadic in their rhesus monkeys. Stinnette and Isaac (1975) gave 2, 4, or 8 mg/kg to squirrel monkeys and reported a decreased response rate on an FI-80 sec schedule at the two lowest doses, but not at 8 mg/kg, when these diurnal animals were tested in the light. (Testing in the dark under caffeine produced a non dose-related depression in responding.)

Davis, et al (1973) administered caffeine-sodium benzoate at 1, 3, 10, and 30 mg/kg intraperitoneally to squirrel monkeys. On a test of physical activity, 3 mg/kg produced very modest increases, 10 mg/kg a moderate decrease, and 30 mg/kg a 40% decrease in traversing a vertical rod. No mention of any toxicity was made at any dose. Several abstracts presented at the 68th FASEB symposium appear to have patterned their dosage selection after this paper.

Caffeine-sodium benzoate (32 mg/kg) elicited immediate "quasi-withdrawal" symptoms in one of three control rhesus monkeys while 4 mg/kg precipitated withdrawal reactions in morphine dependent monkeys (Aceto, et al, 1979). White and Keller (1984) observed a "mimetic" response to injections of 60 mg/kg caffeine that is usually seen only when rats are presented with noxious tastes. Since caffeine is secreted into the saliva, a similar effect might occur in primates given higher doses.

Since the primary function of the caffeine studies is to provide a positive control for the stimulant effects of atropine, one planned dose will be in the adenosine action range of 3-4 mg/kg and one will be a higher dose, 10-16 mg/kg, that is expected to have depressant, but non-toxic effects. Because we will be working with diazepam and a benzodiazepine antagonist later in the project and because these drugs might be used to clarify mechanisms underlying responses we observe to caffeine, one dose should also be in the benzodiazepine antagonist range 0.6-1.0 mg/kg, even though we would not expect to see any behavioral effects of caffeine alone at this dose. The drug will be administered intramuscularly at .16 ml/kg as a caffeine-sodium benzoate solution.

Behavioral Effects of Caffeine.

Activity and Related Behaviors. As noted in the preceding sections, caffeine, at low or moderate doses, will increase the locomotor activity in most, if not all, of the species tested. According to Essman (1971), "...this property resides within the baseline activity levels of the animal" (p. 278); that is, animals with low predrug activity levels will show the effect while those with high baseline levels do not. There is also an interaction between drug effects and circadian variations in activity. Essman (1971) reports that male mice, given 25 mg/kg caffeine, exhibited increased activity during the part of the day when their activity was normally low, but there was a depression when the drug was administered prior to peaks in the daily activity cycle. Essman also describes a finding of particular interest to the present protocol: Isolated mice, while exhibiting slightly higher activity levels than group-housed animals, did not show the expected activity increase when given caffeine just prior to the trough of the daily activity cycle; instead, the drug appeared to reduce activity during this time. Thus, there is a complex interaction between circadian rhythms, social environment, and caffeine. As mentioned earlier, Stinnette and Isaac (1975) found a dose dependent decrease in operant responding with low doses of caffeine (2 or 4 mg/kg) when they tested their diurnal squirrel monkeys in the light when response rates are high. Testing the animals in the dark, when baseline rates at 40% lower, produced a decrease in responding that was independent of dose (2, 4, or 8 mg/kg).

Barry and Miller (1965) utilized a straight alley situation to test different groups of rats under food approach, shock escape, and shock avoidance drives and found that caffeine produced similar increases in running speed under all three conditions. (Procedural errors prevented an accurate assessment of dose-response relationships in this experiment.) Climbing behavior in mice, an apparent escape response, is regularly released by changes in the environment and is inhibited in a dose-dependent fashion by CNS depressants (Kneip, 1960). Using a modifed version of Kniep's climbing test, Bossier and Simon (1967) found no effect at 2 mg/kg, a progressive increase in climbing at doses from 4 to 32 mg/kg, and a decrease with higher doses of 64 and 128 mg/kg. The dose dependent effect was most evident if the mice had previous experience in the situation, suggesting once again the presence of an interaction between base line activity and the drug effect. In this experiment, the highest dose, 128 mg/kg, produced an increase in "exploratory" activity.

Hughes and Grieg (1976) tested rats on ambulation, rearing, and novelty preferences under three doses of caffeine (5, 10, and 20 mg/kg). Ambulation scores were significantly increased by the two lowest doses of caffeine, but returned tc near placebo levels at 20 mg/kg. A decrease in preference for the novel side of the apparatus was found at 10 mg/kg, but this disappeared at the highest dose. The authors note that the regaining of the preference for maximum novelty is inconsistent with the theory that exploratory behavior is inhibited by the heightened arousal produced by CNS stimulants. Caffeine did not affect rearing at any dose level.

Ward, Polan and Geyer (1981) gave graded doses of caffeine to rats and found that doses up to 50 mg/kg increased the startle response to an air puff (121 trials). Augmentation of the amplitude of the response occurred uniformly on all trials rather than specifically affecting habituation to the stimulus. At 100 mg/kg, the drug increased the magnitude of startle for first 20 trials, but decreased it over the rest of the session.

To summarize, it appears that the relationship between caffeine dose and measures of behavioral arousal such as locomotor activity and startle generally takes the form of an inverted U-shaped function. There is a general increase in behavioral reactivity at low to moderate doses, followed by a decline at higher doses. This generalization must, of course, be tempered by recognizing that there may be complex interactions between caffeine and basal activity levels, circadian effects, and the like, as noted at the beginning of this section.

Learning and Performance. With only a few exceptions, experiments on caffeine and learning have looked at the effects of the drug on the performance of learned responses rather than their acquisition. Although an early experiment by Lashley (1917) indicated that caffeine impaired maze learning, dose dependent improvements in acquisition have been reported for a number of tasks in several species. Typically, facilitation is seen with low doses and impairment at high doses. In most cases, it is not possible to determine if the improvement is due to enhanced attention or enhanced formation of associations, or both. Cooper, Potts, Morse, and Black (1969) found that 1 mg/kg of caffeine produced a reduction in trials to criterion in rats learning a T-maze for food reward. Doses of 3 and 7 mg/kg yielded successively smaller, nonsignificant improvements. Castellano (1976) tested mice in a water maze using 0.5, 1, and 2 mg/kg doses. One mg/kg improved both the natural tendency of the animals to swim toward the lighted arm of the maze and their learning to swim toward the darkened arm; 2 mg/kg produced severe deficits on both tasks. Negative results were obtained by Geller, Hartman, and Blum (1971) who gave doses of 1-60 mg/kg caffeine to rats that had failed to learn a lever press discrimination task after six months of training and found that the drug produced no improvement. Stripling and Alpern (1974), using mice, have provided date which suggest that a series of 5 daily injections of caffeine (20 and 80 mg/kg) had a proactive effect on learning a 10-choice maze. Hamsters trained on simultaneous visual pattern discrimination problems learned the more difficult patterns quicker after a dose of 0.5 mg/kg caffeine, but were impaired with 1, 3, an 10 mg/kg. A dose of 0.25 mg/kg had no effect (Rahmann, 1963). Rahmann felt that the optimal dose exerted its effect on the animals' "...concentration and intensity of reaction and hence on learning" (pp 395-396). Retention and relearning were also enhanced by the 0.5 mg/kg dose. Using a classical conditioning paradigm, Wolff and Gantt (1935) found that caffeine lowered the threshold for the appearance of conditioned responses, while Pavlov (1960) described experiments in which the the internal inhibition produced by nonreinforced stimuli was weakened by caffeine.

Although Barry and Miller (1965), as noted above, had found

caffeine increased running speed in escape and avoidance alley tasks, there was no facilitation of learning shuttle box avoidance in either mice (Sansone, 1974) or hamsters (Castellano, C., Sansone, Renzi and Annecker, 1973). In the hamster study, the largest dose, 10 mg/kg, produced a decrement in percent avoidance during the last half of each day's test sessions. In rats, caffeine was reported to improve avoidance responses and increase resistance to extinction, with the largest effect being seen in animals with the poorest learning (Tonini and Babbini, 1961). Similarly, Verhave, Owen, and Slater (1958) reported that caffeine (40 mg/kg) increased the probability of lever press avoidance responding in rats; the effect was most apparent in the poorer performers and in "good" animals that were having a "bad" day.

In an attempt to separate the effects of a generalized increase in responding from effects on the acquisition process, Kulkarni (1972) used a lever press avoidance task in which two levers were present. One bar enabled the animal to avoid shock when a signal was presented; pressing the other had no consequences. Changes in the ratios between correct and incorrect responses were assumed to reflect changes in the acquisition of the discrimination, with an increase in the ratio indicating enhanced learning. Rats receiving 25 mg/kg had higher ratios than those receiving either saline or 50 mg/kg caffeine; those getting 100 mg/kg had significantly lower ratios than controls. There was an inverted U-shaped dose response curve for total responses, with the peak occurring at 50 mg/kg. In 400 trials, the saline group made 199 avoidance responses, the 25 mg/kg group 243, 50 mg/kg 275, and 100 mg/kg 161. The ratio of avoidance responses to responses made on the correct lever during the intertrial interval was identical in the saline and 50 mg/kg groups, significantly higher in the 25 mg/kg and lower in the 100 mg/kg animals. In summary, avoidance learning was enhanced by caffeine at doses lower than those which produced the maximum increase in lever pressing. At the highest dose, performance on the task was significantly impaired although total responses were slightly above control levels. Thus, at least in this experiment, the effects of caffeine on learning were not a simple function of generalized changes in responding.

Experiments on the consolidation hypothesis of memory formation have used caffeine. Pare found that giving 30 mg/kg caffeine within 5 seconds after rats reached criterion on a visual discrimination task produced superior retention when the animals were retested 48 hrs later. (Giving the same dose after either 2 min or 1 hr had no effect.) Castellano's (1976) mice that were learning to swim toward the dark alley of a maze showed significantly faster learning across days when they were given 1 mg/kg immediately after each day's test session. There was no improvement in animals injected two hours after each session. Castellano also gave caffeine to trained animals that had not received caffeine previously and found that 10 and 20 mg/kg doses disrupted the performance of those that were trained to swim to dark (but not that of those experienced in swimming toward light). However, performance always returned to 100% on the day following the administration of caffeine. A more permanent disruption of long term memory was observed in the study by Stripling and Alpern (1974) mentioned above. Injections of caffeine were given once a day for

five days beginning 24 hrs after two days of training in a six-choice brightness discrimination maze. A dose of 20 mg/kg produced impairment on retention tests conducted 24 hrs after the last injection.

In an early paper, Skinner and Heron (1937) found that rats given 10 mg caffeine (approximately 30 mg/kg) increased their responses on a fixed interval (FI) 4-min schedule in resposnse to the drug. Part, but not all, of the effect was attributed to increased hunger since food consumption increased on caffeine days. When given on the fifth day of extinction, caffeine restored the response rates to nearly the level that prevailed during reinforcement. Mechner and Latranyi (1963), using rats in a two lever situation, obtained increased responding on a FI 30-sec schedule in response to caffeine administration; there was considerable interindividual variation in terms of which dose (2.5, 5, 15, or 30 mg/kg) produced the maximum effect, however. In contrast to the effects of two other stimulants, methamphetamine and methylphenidate, the animals maintained the "scallop" in the FI response curve, indicating that caffeine did not disrupt the temporal discrimination of the rats. On a fixed number (FN) schedule, the animals had to accumulate 45 responses on the first lever before a press on the second lever would produce a reinforcer. Caffeine increased the number of responses per reinforcement on the second lever. Once again, there was considerable interanimal variation in the dose that had the greatest effect. On a fixed minimum interval schedule (FMI), which is very similar to a DRL schedule, and on a fixed constant number schedule, the effects of caffeine were inconsistent. Squirrel monkeys served as subjects in performance tests of the effects of several drugs (Davis, Kensler, and Dews, 1973). The caffeine sodium benzoate effects on the FI component of a FI 180-sec FR 30 multiple schedule consisted of a moderate increase in responding at 1 and 3 mg/kg, a peak at 10 mg/kg and a severe depression at 30 mg/kg; the FI scallop was maintained at the peak response dose. The FI data conflict with that of Stinnette and Isaac (1975) whose squirrel monkeys decreased their responding on an FI 80-sec schedule with doses of 2 and 4 mg/kg. The decreases occurred whether baseline responding was high (about 150/hr) or low (about 45/hr).

On the FR component of the multiple schedule of the Davis, et al (1973) experiments, 1 mg/kg caffeine had no effect and increasing doses produced a progressive depression in responding. On a continuous avoidance (CA) schedule (30 sec from a response or a shock to the next shock) all four doses of caffeine produced moderate increases in responses, with the peak at a dose of 10 mg/kg.

Doses of 6 and 12.9 mg/kg enhanced responding in rats on a FI 300-sec FI schedule during the first 30 min of testing; 24 mg/kg decreased response output on this schedule during the third and fourth half hours of the tests (Meliska and Brown, 1982). The increased responding was partially rate dependent, but intermediate control rates were enhanced relatively more by caffeine than the lowest baseline rates. In another study of the effects of caffeine on FI performance in severely food deprived rats, doses of the drug ranging from 3.12 to 50 mg/kg had no effect on performance although the highest dose, 100 mg/kg produced a sharp depression in responding. A similar lack of effect at all but the highest dose was

also found for schedule induced licking and water consumption (Wayner, Jolicoeur, Rondeau, and Barone, 1976). However, when the rats were returned to their free feeding weights, a dose of 3.12 mg/kg produced increased responding and schedule induced licking. (Once again, the 100 mg/kg dose depressed behavior.) The authors discuss their results in terms of the effects of food deprivation without considering that there was probably a rate dependent effect operating. Response rates in the rats deprived to 80% of their free feeding weight were approximately 1000/hr. When they regained normal weight, the control response rates decrereased to 120/hr; 3.12 mg/kg caffeine increased these to about 160/hr. In the Skinner and Heron (1937) paper, the rats were more moderatey deprived (they were allowed two hours of free feeding after each test) and had control rates of about 160/hr; the increases following caffeine were to approximately 250/hr. Wayner, et al also found that tolerance to the 100 mg/kg dose of caffeine appeared after five consecutive daily doses.

Fundaro, Cassone, and Molinengo (1983) found no effect of either 5 or 50 mg/kg caffeine on the transition from a FR to a FI schedule of reinforcement. (Of interest to us, because we will be working with diazepam later in the project, is the report by these authors that diazepam makes it significantly more difficult to make this change in schedule.) Morrison (1969) found no caffeine effect on the reduction in operant responding that is seen when a response is simultaneously rewarded and punished by electric shock.

Performance on a learned successive visual discrimination task (MULT FI [illegible]-sec/EXT schedule) was examined in capuchin monkeys following pretrial administration of 0, 3, 10 and 30 mg/kg caffeine sodium benzoate (Appleby, 1980). Discriminative behavior was enhanced at 10 and impaired at 30 mg/kg relative to sodium benzoate control performance. On a visual search task, humans given caffeine detected more two letter targets than subjects given a placebo. However, when the targets were six letters long, significantly fewer were detected by the caffeine subjects. (Anderson and Revelle, 1983). The authors suggested that the arousal produced by caffeine facilitated the low memory load task (shorter targets) by facilitating attentional processes. Poorer performance on the high memory load task (longer targets) was attributed to arousal hindering short term memory processes. Childs (1978) compared the performance of high coffee users with that of low users given 400 mg caffeine on a visual target-scanning task and found that the low coffee users took considerably longer to complete the task, although accuracy was not affected. On a choice reaction time task, human subjects given 200 mg caffeine took longer to make decisions, but showed facilitation of responding once the decision had been made (Smith, Tong, and Leigh, 1977). Vigilance and reaction time in humans are often improved by doses of caffeine in the 200-400 mg range; however, motor performance and eye-hand coordination may be impaired at moderate to high doses (see Sawyer, et al, 1982, pp 430-431). Davis, et al (1973) tested their monkeys for steadiness and found that caffeine decreased the length of time that the animals could hold a lever away from contact with the edge of a hole.

Sawyer, et al (1982, p 430) have reviewed the evidence that caffeine enhances physical endurance in humans. However, when rats

were tested for endurance on a swimming test, it was found that animals given 20 mg/kg caffeine swam the same distance as controls. They did swim much faster and had significantly shorter swimming times to exhaustion, but there was no evidence of enhanced endurance (Makoc and Vorel, 1974). Squirrel monkeys trained to climb up and down a 260 cm rod for food reward made about 135 complete traverses in 45 min (an animal lifted himself some 350 m in this time) under baseline conditions. This considerable physical effort was enhanced by 3 mg/kg caffeine and depressed by doses of 10 and 30 mg/kg (Davis, et al, 1973).

Social Effects. Sawyer, et al (1982) have reviewed the literature which points to a positive relationship between caffeinism and anxiety and depression in humans. It is not clear whether there is a causal relationship between caffeine and alterations of mood state; if there is, then caffeine might be expected to influence social behavior. In nonhuman animals, changes in locomotor activity due to caffeine might affect social behavior by altering the frequency of social contacts experienced by members of a group. In one study with humans, 1, 2, or 4 mg/kg caffeine decreased two types of aggression while at the same time increasing nonaggressive responding for monetary rewards (Cherek, Steinberg, and Brauchi, 1983). In a study of the effects of caffeine and provocation on aggression, subjects were led to believe they had been given either an arousing drug or a placebo; the subjects used their belief that they had been given a drug as an excuse to release hostility (Ferguson, Rule, and Lindsay, 1982). Although we are continuing to search, our initial review of the caffeine literature has turned up very little on the effects of caffeine on social behavior of nonprimates and nothing on nonhuman primates. As noted in the section on activity, Essman (1971) reported differences between isolated and group-housed mice in their locomotor response to caffeine. Plotnikoff (1962) found that caffeine enhanced the escape response of isolated rats to auditory stress - isolated rats have lower escape responses than normal rats, so this may again be a baseline effect. In one of a series of related papers, the effects of acute and chronic caffeine exposure on play fighting in juvenile rats were examined (Holloway and Thor, 1984). Acute treatment reduced play fighting and increased locomotor activity in a dose dependent fashion, while social investigation was not affected. After 11 days of exposure to caffeine, play fighting increased in animals that ingested an average of 19.6 or 37.5 mg/kg cafeine/day but was the same as controls in animals averaging 150.3 mg/kg/day. Social investigation of a strange rat was reduced by caffeine. Locomotor activity was low in all animals and there were no differences between drug animals and controls. These effects on play fighting are quite similar to those that are produced by d-amphetamine and methylphenidate (Beatty, Dodge, Dodge, White, and Panksepp, 1982). Beatty, et al, suggest that the activation of catecholamine systems is incompatible with the expression of vigorous forms of play, but whether this is caused by a reduced need for social interaction, inhibition of neural mechanisms controlling play, or the activation of competing responses could not be determined. These authors point out that juvenile play fighting is very different from aggression in adults in that in the former, tooth

chattering is absent, biting is inhibited, and stable dominance-submission relationships are rarely formed. In adults, low doses of amphetamine tend to potentiate agonistic behavior and high doses to suppress it. This potentiation interacts with dominance status such that attacks and threats are increased in dominant rats while defense and escape are facilitated in submissive animals. We might expect similar effects in monkeys given caffeine.

Method:

As noted in the statement of the purpose of the work on caffeine at the beginning of this protocol, details of the methods to be used are contained in the original contract proposal and the associated protocol for the study of atropine effects on social behavior and performance.

Drug Administration. As discussed in an earlier section, initially there will be three doses of caffeine sodium benzoate. Depending on the outcome of the first experiments, we can go back and fill in intermediate doses as appropriate, and, if necessary, go to a higher dose. In a pilot study with five young adult males, a dose of 4 mg/kg produced increases in locomotor activity when the animals were observed in a 6 x 6 x 6 ft cage while a dose of 12 mg/ml caused a marked depression in activity. Accordingly, these two doses, plus a dose of 0.8 mg/kg will be used first. The im injections of caffeine-sodium benzoate seemed to act very quickly in the pilot animals. We plan to initiate all behavioral testing, except for the social observations in the animal compounds which requires that several animals be injected on each test, five minutes after administering the drug. In the group social situation, there will be a 20 min lapse between the time the first animal is injected and the time all of the animals are returned to the troop. However, on the tests of dyadic social interactions that will be done in the laboratory, the five min interval between injection and the start of the observations will be enforced. The volume of the injections will be .16 ml/kg in order to restrict the amount of each injection to less than 1 ml. Physiological saline will be used as the vehicle and as the placebo. Drug days will alternate with placebo days except in the case of the highest dose, when two days will intervene between drug administrations. The laboratory normally operates on a five day work week. When this is the case, Monday will normally be a placebo day for all experiments involving tests of performance (e.g., operant, WGTA, and cooperative behavior testing). The caffeine sodium benzoate solutions will be mixed fresh each day and assigned a code by the assistant making up the solutions. Neither the persons administering the injections nor those conducting the behavioral testing will know whether the animals are receiving drug or placebo on any day. While the drug experiments are in progress, each group of animals will be tested or observed for the effects of the drug on only one task per day. It appears that the window for the effects is going to be about 45 min, a duration that is too short for conducting more than one test per day.

Social Observations. Caffeine effects on social behavior will be examined in three sets of adult male M. fascicularis. In the study utilizing T-Troop (one of the two troops containing all age/sex classes of monkeys), the six oldest adult males will be the subjects. On each test day, 3 of the 6 males will be given the drug, the other three will receive injections of vehicle; drug days and placebo days will alternated for each set of three animals until all doses have been given to all subjects. (For this study, an 8 mg/kg dose will be added to the other three doses to provide an intermediate dose between one known to enhance and one known to depress locomotor activity in these monkeys.) The experiment will last eight days. The second group of animals will be the all male troop, I-Troop. In this study, the top ranking and bottom ranking males will receive placebo on all days. The remaining six males will be divided into two sets of three and drug and placebo will be administered according to the same schedule used with the T-Troop males. Social observations will be made in the outdoor compounds with all members of the troops present during the sessions.

Each daily session of social observations of T-Troop will consist of a 10 min group scan followed by 5 min focal observations of each adult male; the session will conclude with another 10 min scan. For I-Troop, the session will begin with a 5 min scan followed by 5 min focal observations of each of the 8 males in the troop; during the focal observations, a continuous scan will also be made; this is possible because of the relatively small number of animals in the group. The order in which the animals are observed in the focal observation procedure is changed each day.

Later, after the animals have been tested with atropine, they will be retested under caffeine during a manipulation of the social structure of the groups. In this procedure, a high ranking animal will be removed from each troop for two weeks and then returned. The effects of caffeine on social behavior during this animal's absence and following his return will be examined using the procedures employed prior to the social manipulation. One or more additional manipulations will be performed if necessary to confirm findings from the first set of manipulations.

The third group of monkeys, known as C-Troop, consists of five young adult males. They are housed in individual cages except when undergoing social annd performance testing. Social testing in these animals consists of daily 5 min pairing of each animal with each of the other four animals in a 6 x 6 x 6 ft indoor cage. In this experiment, a different animal will receive caffeine each day, the remaining four will receive physiological saline. The order of testing of the 10 pairs of animals will be adjusted so that the caffeine animal will complete his four pairings within 40 min after being given the drug. Under this procedure, tests of the effects of each dose will require five days of observations.

Social behavior will be scored and summarized according to the methods described in the original contract proposal. Daily summaries will be used to compare each animal's social behavior under the drug with his nondrugged behavior. Summaries across animals under each dose condition will be used to establish general effects. Other factors to be considered include possible interactions between the drug and the social status of each monkey and between the drug and

the amount of social activity in the troop on a given day (e.g. caffeine might increase some aspects of social behavior on days when the group is relatively quiet, but have no discernible effect on days when activity is high). Should additional tests of caffeine effects on social behavior be needed to confirm or extend the initial findings, seven more adult males, members of a fourth group, NT-Troop, are available for study.

Open Field. The seven adult males in NT-Troop will be used to test for emergence and locomotor activity in the open field using the apparatus and procedures described in the original contract proposal. This will be done twice at all dose levels, including placebo. On the first series of exposures, the animals will be presented with a bare open field; for the second, a novel object (new at each exposure) will be placed in the center of the field. If any animal fails to emerge within five min of the beginning of the test, it will will be gently pushed into the field and observed for the five min test period. For each set of tests, the first day will be a placebo day. Drug and placebo days will be alternated until all doses of caffeine have been given. For the tests with novel objects, all animals will be presented with the same object on the first (placebo) day. Thereafter, the objects will be presented in sets of two. On each day's trials, some of monkeys will be presented with one object of the pair, the others will receive the second object. On the following day, the presentations will be reversed, with each animal being presented with the object it did not see the first day. This will help to control for the fact that the different objects will undoubtedly provoke different degrees of response in the monkeys. If necessary, the study can be repeated with a different set on monkeys from one of the other troops. A tentative hypothesis regarding the effects of caffeine on emergence and locomotor activity is that the intermediate dose of 4 mg/kg will lengthen the emergence latency but increase the amount of locomotor activity once the monkey has emerged. The highest dose should have the oppposite effects and no discernible change in behavior is expected with the 0.8 mg/kg dose.

Complex Problem Solving. Six males from T-Troop will serve as the subjects. They will have been trained on an object quality reversal learning set problem in the WGTA. On these tests, the animals receive four object quality learning set problems each day. Partway through each problem, a reversal is given so that the previously correct object becomes incorrect and vice versa. There are three measures of performance: The number of correct responses prior to the reversal is taken as a measure of habit strength. Correct responses on the second trial of each new problem are an indicant of object quality learning set formation and correct responses on the trial after the trial on which the correct stimulus is reversed is used to assess reversal learning set performance. We expect the effects of caffeine to follow an inverted U function on this task with performance improved only with the intemediate dose. We also expect that performance will be enhanced most in animals that are the poorest performers under normal, undrugged conditions. Normally, Mondays will be placebo days for all animals; subsequently, drug and placebo days will be alternated until all doses have been given.

Should the expected interaction between drug effects and poor baseline performance be obtained, it will probably be worth repeating the study with the same animals in order to confirm the initial results.

Operant Performance. The animals are to be tested on a number of standard operant behavior schedules. The general procedure will be to have the animals achieve stable performance on a schedule and then alternate placebo and drug days until all doses of caffeine have been administered. If necessary, the experiments can be replicated with the same animals in order to verify drug effects obtained with the first series of tests. The following schedules will be used:

(a) *Differential reinforcement of low rate (DRL or IRT)t).* The schedule will have an interresponse time requirement of 18 sec with a 10 sec limited hold. Subjects will be the 7 adult males in NT-Troop.

(b) *Fixed interval (FI).* Animals will be tested on a fixed interval 30-sec schedule under 100% reinforcement and with omitted reinforcement. The subjects will be the 8 males in I-Troop. Although the contract does not specify that the monkeys be tested on a fixed interval schedule, the literature cited above includes a number of studies in which caffeine has produced changes in FI responding. It seems advantageous to test the animals on this schedule since it will allow us to put our work with caffeine in context with that of other laboratories. As soon as the animals complete testing on this schedule, they will be shifted to a random interval schedule as required by the terms of the contract.

(c) *Random interval (RI).* The performance of the I-Troop males on a RI 1-min schedule will be examined under 100% reinforcement and random omission of reinforcement (90% reinforcement).

(d) *Random interval with punishment.* Stable random interval performance will be reestablished under 100% reinforcement after which a punishment condition will be introduced. As we have never worked with footshock with this species, pilot work will be necessary to establish an appropriate level of shock for use in the experiment; the objective will be to produce a significant depression in responding; however, we do not want to suppress responding completely. We will begin with a shock duration of 0.5 sec and an intensity of 0.1 ma and gradually increase intensity across several days until the desired response suppression is achieved. Punishment will be administered by randomly scheduling footshocks two or three times during a test session. When the drug protocol for this procedure has been completed, we will take two of the aniamls and conduct a pilot study to determine the possible utility of a multiple RI - RI PUN schedule for future use. RI will be alternated with RI PUN during each test

session. Under the RI PUN schedule, the animals will be shocked after all responses, whether or not they are reinforced with food. During the time the punishment condition is in effect, a visual cue also will be presented. If the the paradigm proves to be efficient, we may be able to alternate it with the multiple RI · RI schedule which will also be employed with this set of animals. This would allow rapid and efficient assessment of both PUN and behavioral contrast effects.

(e) *Multiple random interval (MULT RI - RI; RI - EXT).* The effects of caffeine on enhanced response rates will be investigated using a multiple schedule, with discriminable RI and RI-extinction components, to produce "behavioral contrast" in which there will be higher rates of response (contrast) in the first component of the schedule when it is alternated with extinction in the second component. RI 1-min schedules will be employed.

(f) *Free operant avoidance.* Tests of drug effects on free operant (Sidman) avoidance will utilize the six 6 adult males from T-Troop. A pilot study will be conducted to evaluate the parameters to be used in the drug study. The interval between footshocks (shock-shock interval) and the time which a lever press will delay a shock (response-shock interval) will be selected to produce an intermediate rate of responding (on the order of 10 responses per minute) so that both response enhancing and response depressing effects of drug manipulations can be detected.

(g) *Fixed Ratio.* There will be two situations in which we can examine the effects of caffeine on performance on a fixed ratio schedule. The five males in C-Troop are to be trained on a FR 12 schedule as a part of the task they will perform in the cooperative behavior study (see below). FR testing will also be conducted in the social group situation as described in the original contract proposal. T-Troop will be used for this work, and all of the animals in the Troop, including the six adult males used in WGTA and free operant avoidance testing, will participate. The drug study will include these males, as well as 4-6 additional monkeys, that will be selected on the basis of both their social status and baseline operant performance. Some of these will be females.

Cooperative Behavior/Performance. In addition to the scoring and analysis of affiliative behaviors that is done on I-Troop, the 24 oldest animals in both T- and NT-Troops, and the 5 males in C-Troop, the C-Troop males are to be tested for performance on a cooperative task. This task is still under development. When it ready, the effects of caffeine on performance will be examined.

Stress. Blood plasma from the subjects will be assayed for stress hormones, including prolactin and cortisol, before and after the animals are subjected to performnce testing under stressful conditions. These conditions include experimental manipulations of

social status and operant tasks which involve footshock and the omission of reward. The assay data will be used to define levels of stress and arousal in the monkeys and we will look for relationships between drug effects on performance and changes in hormone levels.

Schedule:

Most of the male monkeys will be used on more than one behavioral task. As we will also be looking at the effects of atropine on all of these tasks, it will be necessary to examine the effects of both atropine and caffeine on one set of tasks, retrain the animals on their new tasks, and then do the additional drug manipulations with the new tasks. An outline of the schedule to be followed is given below:

TROOP	N	TASK 1	TASK 2	TASK 3	TASK 4
T	6	WGTA	SOCIAL	AVOIDANCE	SOCIAL STRESS
NT	7	DRL	OPEN FIELD-1	OPEN FIELD-2	
I	8	FI	SOCIAL	RI; RI PUN	MULT RI EXT
C	5	DYADIC SOCIAL	FR	COOPERATION	
T	ALL	SOCIAL	GROUP FR	SOCIAL STRESS	

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